Function of social calls in Brown Long-eared bats *Plecotus auritus*

by

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Author and Brown Long-eared bat *Plecotus auritus* at a maternity roost site in East Sussex May 2007.

This thesis is dedicated to my mother Nuala Burnett

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Summary: Function of social calls in Brown Long-eared bats *Plecotus auritus*

Microchiropteran bats produce vocalisations for two purposes: echolocation and communication. Vocalisations used for communication are often referred to as social calls. In this thesis I examined the nature of Brown Long-eared bats *Plecotus auritus* social calls recorded at roost and foraging sites through a combination of recording and playback experiments.

A total of 11,484 social calls were recorded at 20 maternity roosts sites and three types of vocalisations were identified on the basis of shape, referred to as Type A, B, and C. Although Type A vocalisations shared the same basic pattern, it was a very large group within which there was a lot of variation in acoustic parameters. Principal component analysis and model-based cluster analysis were used to look for patterns within this group, and this identified six clusters. Maternity colonies surveyed in this study varied in size from as few as nine up to 98 bats, and the number of social calls recorded at the roost sites was highly correlated with the numbers of bats present in the colony. The analysis of seasonal patterns of social call production revealed that the number of social calls recorded at maternity roost sites showed a linear increase from June to September, whereas, the number of bats emerging decreased sharply from August to September.

Simulations of *P. auritus* social calls were used to investigate behavioural responses to calls away from roost sites using the Autobat. *P. auritus* were clearly much more responsive to simulations of their own species' social calls than to the other stimuli tested. This strongly suggests that the responses to the Autobat represent attempts to interact with the source of the stimulus. Recording with ultrasound and infra-red video was conducted to test the bats' responses to the different types of synthesised call and whether these responses varied seasonally.

A female's approach response to the stimulus may represent an attempt to repel a perceived intruder from her foraging area. Alternatively, if calls were used to coordinate foraging by advertising the location of resources to other females that share the range, a response may represent an attempt to move towards such resources. Experiments showed that females were significantly more likely to respond to a stimulus produced within their core foraging area, than in the peripheral area, or outside their foraging area. On the other hand, while females regularly shared foraging ranges with other females, there was little evidence of co-ordination of movements between simultaneously radio-tracked dyads. It was concluded that responses to the stimuli probably represent attempts to repel perceived intruders from the foraging area.

The thesis concludes with a discussion of some of the advantages and limitations of using play-back of synthesised social calls in the field to investigate vocal communication in bats. Ways in which studies of captive bats of known relatedness could be used to further elucidate the functions of social calls are discussed.

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1.1 - Animal communication

Animal communication can be defined in many ways but, essentially, it involves the transfer of information from one animal to another. An exchange of information can either occur intra-specifically (between individuals of the same species) or inter-specifically (between members of different species). This may, or may not, involve a specific behaviour or signal, and the receiver may, or may not, act upon the information received. Animals use a variety of signal types, which can either be olfactory, visual or auditory in nature to communicate information such as territory ownership (Cooney and Cockburn, 1995; Katti, 2001; Hurst and Beynon, 2004), the presence of predators (Schwagmeyer, 1980; Macedonia, 1990) or to advertise the detection of a food source for species that co-operatively hunt (Bednarz, 1988; Bshary et al., 2006).

1.2 – Vocal communication

Vocal communication, communication produced via the vocal apparatus, is a common communicative signal used by a variety of different animals, for example, birds, anurans, elephants, canids, primates and bats. The production of vocal signals can sometimes have significant associated costs for the individual emitting the signal. For example, the rate at which fringe-lipped bats *Trachops cirrhosus* capture frogs in the Neotropics is significantly higher when the frogs are producing calls to attract females (Tuttle and Ryan, 1981). Male tungara frogs *Physalaemus pustulosus* produce several mating call variants and one call in particular, the 'chuck' call is preferred by females. However, emitting a chuck call makes a male *P. pustulosus* much more vulnerable to predation by *T. cirrhosus* (Ryan et al., 1982).

Not only are there inherent risks associated with behaviour that increases conspicuousness of individuals to predators, it has also been demonstrated that, for some animals, there is an energetic cost in producing the vocal signal. For example, it has been shown that oxygen consumption in the gray treefrog *Hala versicolor* is 21 times greater than consumption at resting (Taigen and Wells, 1985). There are also significant energetic costs in some loud calling mammals, such as red deer *Cervus elaphus* (Cluttonbrock and Albon, 1979; Reby and McComb, 2003). However, the energetic cost of vocal production may be less significant for other animals, such as songbirds (Oberweger and Goller, 2001).

The costs of vocal communication are offset by the benefits (to the individual). These benefits, which are not mutually exclusive, include attracting mates (Catchpole et al., 1984; Buchanan and Catchpole, 1997; Buchanan and Catchpole, 2000), advertising ownership and deterring competitors (Goldberg and Ewald, 1991), broadcasting information on physical quality (Cowlishaw, 1996), broadcasting social information (Leong et al., 2005), broadcasting information about the physical environment (Wrangham, 1977; Hauser and Wrangham, 1987; Wilkinson and Boughman, 1998) and maintaining contact with or recruiting social companions (McComb et al., 1994; Grinnell et al., 1995; Grinnell and McComb, 1996).

1.3 - Communication in microchiropteran bats

Microchiropteran bats produce vocalisations for two purposes: echolocation and communication. For echolocation, pulses of ultrasound are emitted through the mouth or through the nose and returning echoes are interpreted by the bats (Griffin, 1958; Metzner,

1991; Speakman, 2001) which allows them to estimate the distance and location (or the direction of flight) of prey (Neuweiler, 1989) and to navigate through their environment (Holland, 2007; Holland, 2009). Bats also produce communication calls, whose primary function is thought to be communication with other bats, and these are often referred to as social calls

A great deal of research has been carried out on the function of echolocation calls in bats (Jones, 1995; Obrist, 1995; Arita and Fenton, 1997; Schnitzler and Kalko, 2001) and call structure has been described for a variety of species (Fenton and Bell, 1979; Fenton and Bell, 1981; Waters and Jones, 1995; Waters et al., 1995; Vaughan, 1997b; Jones et al., 2000; Russo and Jones, 2002). In contrast, much less is known about the characteristics of the social calls of bats. This may be in part because social calls are given much less frequently than echolocation calls, which are typically produced continuously during flight and, as a consequence, are recorded by researchers less often. Even when social calls are produced frequently in certain situations, they are more specific to particular contexts than echolocation calls. Furthermore, social calls are often emitted in situations where it is difficult to ascertain the social context in which the call was produced (Brown et al., 1983; Kanwal et al., 1994; Andrews et al., 2006; Ma et al., 2006), and it is often not possible to determine which individual is producing the call. Other studies have described the context in which social calls were given (Barclay and Thomas, 1979; Aldridge et al., 1990; Andrews et al., 2006), but have been unable to assign function to call type, due to the inherent difficulties in studying communication in small, fast-flying nocturnal mammals.

1.3.1 - Echolocation

Sound plays a vital role in the lives of Microchiropteran bats, all of which have the ability to echolocate. Echolocation and social calls can be distinguished in terms of their primary function, but there is some overlap. The echolocation calls of any bat have the potential to communicate (intentionally or unintentionally) its identity, location and activity. For example, a study by (Barclay, 1982) on little brown bats *Myotis lucifigus* found that echolocation calls can influence the behaviour of conspecifics and bats of other species. Barclay (1982) used field playbacks of echolocation calls from *M. lucifigus* and attracted conspecifics from up to 50 metres away. *M. lucifigus* was also attracted to playbacks of the echolocation calls of big brown Bats *Eptesicus fuscus* and vice versa. Subadults of *M. lucifigus* responded more to playback of echolocation calls than adults. These results suggest that conspecifics at least, and perhaps other species, may eavesdrop on other bats to locate potential foraging sites (Barclay, 1982).

Furthermore, it has also been demonstrated for a variety of species, including the *E. fuscus* (Kazial et al., 2001; Kazial and Masters, 2004), *M. lucifigus* (Kazial et al., 2008a; Kazial et al., 2008b; Melendez and Feng, 2010), greater mouse-eared bats *Myotis myotis* (Yovel et al., 2009) and the intermediate leaf-nosed bat, *Hipposideros larvatus* (Jiang et al., 2010) that echolocation calls can carry specific signatures that can be used for the recognition of conspecifics and, also, for discriminating between familiar and unfamiliar individuals. For example, playback experiments with lesser bulldog bats, *Noctilio albiventris*, in which individuals were presented with calls of familiar or unfamiliar conspecifics, cohabitant or noncohabitant heterospecifics and ultrasonic white noise as a control, found that bats reacted

with a complex repertoire of social behaviours and the intensity of their response differed significantly between stimulus categories. Stronger reactions were shown towards calls of unfamiliar conspecifics than towards heterospecifics or white noise (Voigt-Heucke et al., 2010).

1.3.2 - Social Calls

Social calls typically differ from echolocation calls in a number of acoustic characteristics. Social calls may be harsh and broadband or tonal and broadband, as described for species such as *M. lucifigus* (Barclay et al., 1979) and are often much longer in duration than echolocation calls, with low frequency components and multiple syllables (Fenton, 1994; Altringham and Fenton, 2003). Although both communication and echolocation calls are of laryngeal origin, there is evidence that separate vocally active brainstem areas are involved in the functional control of communication and echolocation calls (Fenzl and Schuller, 2007). To maximize the distance over which a signal can be heard, communicating bats tend to use lower frequency sounds to minimise the impact of attenuation (Lawrence & Simmons, 1982). For example, the heart-nosed bat *Cardioderma cor* produces short, low intensity echolocation dominated by high frequency sounds (>20kHz), which limits the range over which the calls carry (Vaughan, 1976). The song calls of *C. cor* are much lower in frequency (<20kHz) and therefore are audible over a greater distance. The use of song is seasonal produced when *C. cor* fly about their feeding areas. Feeding success appears to be related to singing at times of low food abundance (Vaughan, 1976), suggesting that it may serve a role in mutual avoidance.

Much of the research into bat vocal communication has focused on vocalisations given in a few specific contexts, such as mating (Barclay and Thomas, 1979; Thomas and Fenton, 1979; Keeley and Keeley, 2004) or during maternal reunion with offspring (Balcombe, 1990; McCracken and Gustin, 1991; Balcombe and McCracken, 1992; Defanis and Jones, 1995; Knornschild and Von Helversen, 2008). Bat also produce social calls at resting places such as roost sites (Chaverri et al., 2010), swarming sites (Parsons et al., 2003) and hibernation sites (Andrews et al., 2006). A number of additional functions proposed for the social calls of bats include:

i. Defence of resources: in areas of high insect density pipistrelle species have been observed foraging in groups of up to 40 individuals without any signs of intra-specific aggression. However, at low insect densities, there was intra-specific aggression (Racey and Swift, 1985). In both *Pipistrellus pipistrellus* and *Pipistrellus pygmaeus* rates of social call production increase as insect density decreases and there is a significant reduction in activity by conspecifics during playback of social calls (Barlow and Jones, 1997). This suggests that food resources are being defended and that social calls have an agonistic function towards other individuals of the same species when insects are scarce.

ii. Group cohesion: Wilkinson and Boughman (1998) found that greater spear-nosed bats *Phyllostomus hastatus* give loud, broad-band 'screech' calls when foraging and these calls appear to function as contact calls that recruit and co-ordinate foraging among group members.

iii. Mobbing: captured *Pipistrellus pipistrellus* emit distress calls that are colony-specific and may attract conspecifics to 'perform mobbing behaviour as an anti-predator response' (Russ et al., 1998).

iv. Attracting mates: (Behr and von Helversen, 2004) found that courtship songs were exclusively observed when male *Saccopteryx bilineata* displayed towards a female *S. bilineata.* In contrast to territorial songs (which were short, rather stereotyped and not obviously directed towards a certain conspecific), courtship songs were long and complex and consisted of highly variable elements ("calls").

v. Territorial male interactions: during the spring, males *Pipistrellus pipistrellus* start setting up individual territories around day roosts. Intruders into the territory are chased away by the resident male, who produces calls that are presumed to be agonistic (Lundberg and Gerell, 1986).

Each of the studies listed above focused on a particular vocalisation type produced in a specific context. However, it is most likely that each species of bat produces a variety of calls that differ in function (Pfalzer and Kusch, 2003). A small number of studies have examined multiple vocalisations within a species and have revealed an extensive repertoire of vocalisations (Barclay et al., 1979; Kanwal et al., 1994; Ma et al., 2006; Melendez et al., 2006) with evidence of syntax (Kanwal et al., 1994; Bohn et al., 2009) and of call composition that varied according to the behavioural contexts in which they were emitted (Bohn et al., 2008). Such variation in vocalisation potentially allows the communication of information concerning the sender and/or situation. If vocalisations are associated with different behavioural contexts, they are likely to have different meanings that should be reflected in their acoustics (Bohn et al., 2008).

1.4 - Approaches to studying communication in animals

One method employed by researchers to investigate the function of vocalisations is to record the sound and play it back to the target species, and examine the response. The use of playback provides a powerful tool for investigating cognitive abilities in animals. Replaying recordings of vocal signals may reveal what information is contained in the call (McGregor, 1992). For example, a study on the female copulation calls of barbary macaques *Macaca sylvanus* revealed that males can discriminate female copulation calls given at different stages of the oestrus cycle. Playback of female calls revealed that males responded more strongly (by looking at or approaching the loudspeaker) around the time when conception was most likely to occur, in comparison to their responses to female calls given around the time the time conception was least likely to occur (by looking away or ignoring the loudspeaker). This suggests that the calls of *M. sylvanus* may contain information on reproductive state, which males can perceive and use in such a way as to increase their reproductive success (Semple and McComb, 2000).

1.5 - Approaches to studying communications in bats

Playback experiments have been used to investigate behavioural responses of bats to a variety of different call types, both echolocation (Barclay, 1982; Voigt-Heucke et al., 2010) and social (Fenton et al., 1976; Barlow and Jones, 1997; Russ et al., 1998; Wilkinson and Boughman, 1998). Playback provides a tool to facilitate the study of bat social calls when it is not practical to study the emission of social calls. However, playback of ultrasound is problematic in that the equipment required for recording and reproducing ultrasound frequencies is expensive and fragile for regular use in the field (Hill and Greenaway, 2005). Furthermore, it can be difficult to obtain good quality recordings of social calls, because they

are given infrequently, and there is often background noise. In order to address this problem Hill and Greenaway (2005) developed an acoustic lure, in the form of a portable ultrasound synthesiser, the Sussex 'Autobat', which can be programmed to emit simulated bat social calls. The lure was initially developed to attract elusive woodland bats, such as Bechstein's bat *Myotis bechsteinii*, and field studies demonstrated that broadcasting simulated social calls at woodland sites attracted bats to mist nets and significantly increased captures rates (Hill and Greenaway, 2005; Hill and Greenaway, 2008).

Preliminary fieldwork at a variety of woodland sites showed that bats responded well to the Autobat, and that brown long-eared bats *Plecotus auritus* responded particularly well to simulated social calls. The Autobat output is modelled on bat social calls, but the repetition rates are much higher. So one of the central questions was 'why do bats respond'? Also, what, if anything, can this tell us about the function of social calls? It would be totally impractical to investigate the function of social calls by playing them at anything approximating natural rates, because these are so low, that it would be difficult or impossible to obtain sufficient results. Preliminary fieldwork also suggested that bats were attracted to a range of stimuli, modelled on social calls, so an alternative was to investigate the bats' responses to these, whether responses to different stimuli differed, and whether responses varied in relation to spatial and seasonal context.

1.6 - The study species brown long-eared bat *Plecotus auritus*

1.6.1 - Distribution

The brown long-eared bat (*Plecotus auritus*, Linnaeus 1758) is a medium-sized Palearctic vespertilionid with a widespread distribution. *P. auritus* range in Europe extends westward to the west of Ireland (Shiel et al., 1991), as far south as central Spain (Fernandez, 1989) and central Italy (Crucitti, 1989), and as far north to Sweden (where the northern edge of the range appears to be 63^0N) (Ahlen and Gerell, 1989). It was also thought that *P. auritus* extended eastwards as far as Sakhalin and Japan (Corbet and Hill, 1991) and in Asia it was found in Mongolia and north-east China (Zheng and Wang, 1989). However, a recent study using a combination of morphological and molecular data revised the genus and concluded that *P. auritus* is restricted to Europe including the Ural and Caucasus mountains (Spitzenberger et al., 2006). *P. auritus* is common and widespread in Britain and is distributed throughout the country except in the far north and north-west of Scotland and offshore islands (Swift, 1991). It has been recorded in a variety of habitat types but is usually associated with woodland or parkland.

Two species from the genus *Plecotus* occur in Britain; the brown long-eared bat *P. auritus* (Linnaeus 1758) and the grey long-eared bat (*P. austriacus*, Fischer 1829). The two species are morphologically very similar but can be distinguished through a set of measurements used in combination (Stebbings, 1967; Greenaway and Hutson, 1990; Swift, 1998). The two species occur sympatrically in central Europe, but *P. austriacus* is generally found at lower altitudes and latitudes than *P. auritus* (Swift, 1991; Altringham, 2003). In Britain *P. austriacus* is at the northern edge of its distribution. It is one of the rarest British bat species,

and only found along a thin strip of southern England from the coastal areas of Devon to West Sussex (Altringham, 2003). They are more common on the Isle of Wight and the Channel islands.

1.6.2 - Morphology

P. auritus weighs approximately 6 – 12 grams in weight, with a wing span of 230 – 285mm, forearm length of 34 – 42mm and a body length of 37 – 48mm. Species of the genus *Plecotus* are characterised by their long ears, with pinnae length of 29-38mm, which is well over half that of the body (Swift, 1991; Swift, 1998). The wings are short and broad with a low aspect ratio and low wing loading (Norberg and Rayner, 1987). The wing shape of *P. auritus* allows for slow, fluttering, manoeuvrable flight in cluttered habitats (such as woodland) (Aldridge and Rautenbach, 1987; Sevcik, 2003). It frequently hovers (Entwistle et al., 1996) and can rise vertically for a few feet (Norberg, 1976) and this enables the species to forage by gleaning (capturing prey that is crawling or at rest on solid surfaces rather than in flight). The large ears can pick up return echoes from short low intensity calls (Waters and Jones, 1995) and this species also uses passive listening of prey–generated sounds to locate prey (Anderson and Racey, 1991; Anderson and Racey, 1993). Laboratory experiments have also shown that *P. auritus* use visual cues for detection, but exploit additional information, such as echolocation and passive listening, during the final pursuit (Eklöf and Jones, 2003).

1.6.3 - Annual cycle of behaviour and reproduction

Plcotus auritus displays a seasonal cycle of behaviour in common with other temperate bat species. Colonies form in spring and last throughout summer, with autumn and winter mating, sperm storage and delayed fertilisation (Swift, 1998). When *P. auritus* emerges from hibernation in the spring, the bats spend time in transient roosts before the females come together to form maternity colonies in the late spring early summer. However, *P. auritus*, is unusual among temperate zone bats in that summer maternity colonies include adult males as well as females (Speakman et al., 1991; Entwistle et al., 2000; Burland et al., 2001). Estimates of colony size vary with some researchers on the continent estimating as few as 5 – 10 on average (Horáček, 1975), to estimates of over 80 females for some colonies (Swift and Racey, 1983; Battersby, 1999).

Sexual maturity begins for a few individuals (5 – 10%) in the first autumn but, for most *P. auritus* sexual maturity is attained in their second year (Speakman and Racey, 1986; Entwistle et al., 1998). The first mating can be observed in mating roosts in August and the last in spring gathering roosts in April (Furmankiewicz, 2002; Furmankiewicz, 2008). The gestation period is between 60 and 70 days (Swift, 1981; Speakman and Racey, 1987) and a single young is produced in June or July (Swift, 1981). The juveniles become volant at around 30 days and are usually fully weaned at six weeks (Swift, 1981). Hibernation begins from around the end of October through until April but bats can emerge to feed and drink throughout the winter (Hays et al., 1992).

1.6.4 - Roosts

In parts of central Europe, *P. auritus* are known to roost in tree cavities (Horacek, 1975), and it seems likely that these are the natural roost sites for the species (Swift, 1998; Dietz et al., 2009). In Britain, summer maternity colonies are most frequently found in buildings (Stebbings, 1966; Jones et al., 1996; Entwistle et al., 1997; Battersby, 1999), but bat boxes (Boyd and Stebbings, 1989) and tree roosts (Murphy *et al.* in prep) are also commonly used, particularly in southern Britain. Temperature has been shown to be an important factor in summer roost selection (Entwistle et al., 1997; Battersby, 1999).

Plecotus auritus hibernates in a variety of structures including buildings, underground sites such as caves, well pits and railway tunnels and, also, in tree holes (Swift, 1991). *P. auritus* is a non-migratory species with the longest recorded movement being 90km (a female from Germany noted in Dietz et al., 2009).

1.6.5 - Foraging

The wing and ear morphology, coupled with low intensity echolocation, suggest that *P. auritus* is adapted to feeding in cluttered habitats (Norberg and Rayner, 1987) and studies have shown that the species feeds predominately in woodland (Entwistle et al., 1996). *Plecotus auritus* emerge from their day roosts fairly late relative to many other verspertilionid species and this late emergence is almost certainly connected with their habit of gleaning (Rydell et al., 1996; Swift, 1998). The time of emergence in insectivorous bats is likely to be a compromise between the need to emerge to feed and avoiding the risk of predation at high light levels (Speakman, 1991; Jones and Rydell, 1994). *Plecotus auritus* usually use

landscape features, such as hedges or tree-lines, to fly between day roosts and foraging sites and tend to avoid flying in the open (Entwistle et al., 1996). This may reduce the risk of predation by nocturnal birds, such as tawny owls (Speakman, 1991; Lesinski et al., 2009a; Lesinski et al., 2009b), barn owls (Speakman, 1991; Petrzelkova and Zukal, 2003) and birds of prey, such as hawks or raptors, flying at dusk (Speakman, 1991; Fenton et al., 1994). *Plecotus auritus* has been described as a woodland bat and occurs in a variety of woodland types including deciduous (Entwistle et al., 1996), coniferous (Fuhrmann and Seitz, 1992) through to birch scrub and gardens with mature trees (Swift and Racey, 1983). It has also been recorded in orchards and parkland among meadows (Barataud, 1990). Research in Germany and Sweden recorded frequent use of conifer forest (Fuhrmann and Seitz, 1992; Ekman and DeJong, 1996), while in north-east Scotland the species showed a significant preference for deciduous, broadleaved woodland, and utilised only the edges of conifer plantations (Entwistle et al. 1996).

1.6.6 – Diet

Early studies based on insect remains found under feeding perches suggested that *P. auritus* was a moth specialist and consumed little else (Poulton, 1929; Buckhurst, 1930). However, more recent investigations, based predominately on faecal analysis, have shown that the species is flexible in its diet and takes a variety of prey including non-flying arthropods such as Araneae and Dermaptera (Swift and Racey, 1983; Rydell, 1989; Shiel et al., 1991; Vaughan, 1997a). The remainder of the diet consists of moths, Coleoptera and Diptera (Vaughan, 1997a). Prey items may also be caught inside the roost, both in summer roosts (Swift and Racey, 1983; Rydell, 1989) and winter hibernation sites (Roer, 1969). Feeding perches are often used to consume larger prey items (Thompson, 1982; Robinson, 1990).

1.6.7 - Conservation status

Plecotus auritus is one of the more common bat species in the British Isles and parts of Central Europe, with a population estimate of 245,000 individuals in Britain (Battersby, 2005). The 2009 IUCN Red List (IUCN, 2009) classifies the species as "Least Concern" and the population trend as "Stable". However, the National Bat Monitoring Programme reported an estimated 20% decrease for this species in the UK over a period of seven years (BCT, 2004), which led to its inclusion in the revised List of Priority Species for the UK Biodiversity Action Plan (B.R.I.G., 2007).

1.6.8. - Echolocation and social calls

P. auritus is sometimes described as 'the whispering bats' as their echolocation calls are characterised by their quietness with a fast pulse rate (Russ, 1999). The call is a short FM sweep, approximately 2ms in duration, with a fast pulse rate that can be heard most clearly on a detector set between 30kHz and 40kHz, but it is very faint and only detectable if the bat is less than about five metres away (Swift, 1998).

Ahlén (1981) identified a loud call detectable at 40 metres or more, that was longer in duration (7.1ms) and descended in frequency from about 42kHz to about 12kHz, ending with a short (1ms) CF portion at about 12kHz, which was also the frequency of maximum energy followed by a short downward FM sweep (Ahlen, 1981). Ahlén (1981) noted that this call type was emitted intermittently when *P. auritus* was flying inside barns and mines and more regularly when flying in the open. Coles *et al.* (1989) demonstrated that this peak frequency of 12kHz coincided with the centre of the most sensitive range of *P. auritus* hearing and

proposed that this call could be a long, distance communication call (Coles et al., 1989). However, the function of this call was not determined.

Simulation of *P. auritus* social calls using the acoustic lure represents a novel approach for investigating the function those calls. For example, if *P. auritus* social calls away from roost sites are used to defend resources, then it would be expected that *P. auritus* females would respond more frequently to calls broadcast within their foraging range compared to calls broadcast outside of their foraging rage. Alternatively, it could be that social calls away from the roost sites are used in long distance communication to co-ordinate foraging behaviour, then it would predicated that there would be evidence of co-ordinated foraging behaviour amongst colony members.

P. auritus regularly emit social calls at summer roost sites around the time of entering and exiting the roost (Entwistle, 1994; Battersby, 1999) and during dawn swarming at roost sites. It is likely that some of the calls emitted at roosts sites will be in the context of maternal care. Isolation calls (i-calls) emitted by infant *P. auritus* have been shown to have individual variation, and laboratory experiments demonstrated that vocal signatures allowed adult females to recognise and suckle their own pup (Defanis and Jones, 1995). However, *P. auritus* also emit also social calls at roost sites during gestation, which cannot be related to maternal care. Alternative hypotheses would be that these calls are related to processes such as group cohesion or information transfer. If social calls at roost sites were a function of group cohesion, then it would be expected that the number of calls would be positively correlated with the number of bats in the roost and there would be evidence of variation in social call structure at roost sites.

Furmankiewicz (2004) reported that *P. auritus* also emit social calls at swarming sites in Continental Europe in spring (March-April) and in autumn (September-October) and speculated that these calls could function as mating calls. However, the frequency of occurrence of *P. auritus* at swarming sites in the UK seems to be less than on the continent (Furmankiewicz, 2002; Parsons et al., 2003; Veith et al., 2004; Furmankiewicz and Altringham, 2007) and alterative mating sites (such as roosting locations near to or within disbanded maternity colonies), may provide male bats with more regular access to females. If social calls at roost sites were related to mating, then it would be expected that the number and variation of calls would increase in the mating season.

1.7 - The study area

The study was conducted at various woodland, village and farm sites across the counties of East and West Sussex, southern England. Sussex is one of the most wooded counties in Britain with about 15% of the county covered by woodland (Oakes and Whitbread, 1988). Sussex is also one of the warmest counties in Britain with a mean minimum temperature of approximately 3^{o}C, and mean maximum temperature of approximately 20^{o}C. Mean annual rainfall ranges from 800mm along the coast to 1200mm on higher ground (MetOffice, 2010). Sussex is a relatively densely populated county with a population estimate of 1.38 million people at the last 2001 census. This represents an estimated population rise of 7% since the census of 1991 (National Statistics Office, 2010) and many rural towns have expanded, reducing the amount of semi-natural habitat available in the county.

1.8 - Specific aims and objectives

The overall aim of my research was to examine the nature of social calls of brown long-eared bats *Plecotus auritus* and improve and expand our knowledge of their functions and characteristics. *P. auritus* produce social calls frequently at roosting sites and much less frequently when foraging. This thesis will examine the nature of social calls recorded at roost sites and responses to them in the field through a combination of recording and playback experiments. At the same time the study will examine aspects of the behavioural ecology of the species, specifically relating to ranging behaviour, nocturnal activity patterns and patterns of maternity colony use.

Specific objectives of my study were as follows:

1. To describe the structure and variability of *P. auritus* social calls in terms of their acoustic parameters;

2. To examine temporal patterns of social call production at summer roosting sites;

3. To describe behavioural responses of *P. auritus* to simulated social calls;

4. To assess the extent to which sex and season influence the responses of *P. auritus* to simulated social calls;

5. To determine whether responsiveness to simulated social calls varies according to the location of the stimulus in relation to a female's foraging area; and

6. To assess the extent to which females occupying adjacent or overlapping ranges coordinate their ranging movements.

In order to address these objectives I present, in Chapter 2, an analysis and classification of the social calls of *P. auritus* recorded at 20 maternity roost sites across three geographic locations. In Chapter 3 I go on to describe the patterns of social call production at the 20 maternity roost sites, in relation to the size of the colony and, also, the time of day and season. In Chapter 4 I assess the difference in behavioural responses to a variety of stimuli, and how responses to simulations of *P. auritus* social calls vary seasonally. Chapter 5 describes a pilot study used to develop a methodology for a radio-tracking experiment, to examine responses of *P. auritus* and the subsequent radio-tracking study to establish home range use by females. Chapter 6 details a preliminary study to determine reliable distances at which *P. auritus* respond to social calls and the trialling of a system to indicate the approach of a radio-tagged individual. I assess whether the propensity of *P. auritus* to respond to stimulus calls is dependent on the stimulus location in relation to foraging area. Patterns of range use and interactions between simultaneously radio-tracked pairs of female *P. auritus* are investigated in Chapter 7. The thesis concludes with a general discussion in Chapter 8.

2.1 - Introduction

Classification of the kinds of signals animal use is an important step towards understanding their communication. Classification of vocal signals has been carried out fairly extensively for a variety of different species of birds (Weary 1990, Armstrong 1992, Horning et al., 1993; Burnell and Rothstein, 1994; Ficken et al., 1994; Nowicki et al., 1994; Gentner and Hulse, 2000; Baker and Logue 2003; Ranjard and Ross, 2008), cetaceans (McCowan, 1995; Janik, 1999; Rendell and Whitehead 2003; Boisseau, 2005; Shulezhko and Burkanov, 2008; Soldevilla et al., 2008), and primates (Mitani and Marler 1989; Ambrose 2003; Becker et al., 2003; Rukstalis et al., 2003; Miller and Hauser, 2004; Savigh et al., 2007; Gamba and Giacoma, 2007; Chakladar et al., 2008; Pozzi et al., 2010). Studying the vocal communication of bats presents a particular challenge, compared to most birds and other diurnal animals, because of their nocturnal habits, often inaccessible roosts and the ability to fly. These factors are likely to have hindered the study of communication in bats in their natural habitat.

A number of studies have described the signal structure of various types of communication calls in a given context. Examples include infant isolation calls (Gelfand and McCracken, 1986; Defanis and Jones, 1995; Knornschild and von Helversen, 2008), mating calls (Barclay and Thomas, 1979; von Helversen and von Helversen, 1994; Behr and von Helversen, 2004), and group contact calls whilst foraging (Wilkinson and Boughman, 1998). A few studies have investigated multiple vocalisations within a species. These studies have shown that for some species of bats communication calls may consist of several variations and combinations of CF and FM signals (Kanwal et al., 1994; Pfalzer and Kusch, 2003; Andrews et al., 2006; Ma et al., 2006; Knornschild et al., 2010) and may include evidence of syntax (patterns in the

sequences of calls given) (Kanwal et al., 1994; Bohn et al., 2008). Furthermore, research has shown that some species of bat are capable of vocal learning (Esser, 1994; Boughman, 1998).

Many studies investigating bat social call structure have described individual elements of the 'calls' through visual examination of sonograms (Fenton, 1976; Barclay et al., 1979; Barclay and Thomas, 1979; Pfalzer and Kusch, 2003; Furmankiewicz, 2004; Jahelkova et al., 2008). The acoustic parameters of a call may be important for conveying information, but there are other features of vocalisations that may also be of importance, i.e. the temporal distribution of call emission, whether calls are emitted singly or in distinct clusters (Kanwal et al., 1994; Bohn et al., 2008), how frequently calls are emitted over time (Hauser, 1998), and whether calls are emitted as sequences (Bohn et al., 2009). Furthermore, visual inspection of calls with the aim of classifying them into separate groups, based on spectrogram structure alone, is generally qualitative, difficult to conduct on large datasets and, therefore, subject to the individual interpretation of the researchers (Melendez et al., 2006).

To understand the acoustic communication of a species it is essential to distinguish between different call types. As animal communication is likely to be influenced by the context in which it occurs, it is useful, in as a first step to focus on a single context. This chapter presents the results of acoustic analysis of *P. auritus* social calls produced in the vicinity of a number of maternity roosts is Sussex. Calls were recorded continuously through the night, and in each of the five summer months. The aims of this chapter are to describe the structure of social calls recorded at roost sites and to employ statistical methods to objectively classify the calls.

2.2 - Methods

2.2.1 - Recording calls

An automated ultrasound recorder was placed at the main exit location for emerging *P. auritus* at 20 maternity roost sites. All ultrasound was recorded from dusk to dawn, on a monthly basis from May through to September, at each of the 20 locations (see Chapter 3 for more detailed roost information and patterns of social call production). Automated remote ultrasound detectors allow large amounts of data on bat presence and vocal activity to be collected. This study used a custom-built ultrasound monitoring device, called the Ultrabat, shown in Figure 2.1, that records data directly onto a hard disk. Once the Ultrabat has been triggered by a bat call, or other ultrasound, it records for a preset period of three seconds. Once the three second sampling period has finished, there is a 10ms interval delay to allow the record to be written to the file. The file is then stored on the hard disc along with the time and date that the file was created. The Ultrabat produces high quality recordings that are suitable for quantitative analysis using sound analysis software. The microphone within the Ultrabat system is a Knowles EK2132 model. This broadband microphone is used commonly by many standard commercial bat detector models (such as Petterson time expansion detectors & Wildlife acoustics SM2). However, the frequency response of this microphone was measured (see Appendix A.4.3.2) and it has low frequency roll-off and does not record frequences below about 10kHz, which means that parameters of the call below this frequency are not captured accurately. Unfortunately, social calls do seem to have low frequency components that my recordings did not capture.

2.2.2 - Analysis of calls

The most common techniques currently available to convert the ultrasonic vocalisations of bats into audible sound include frequency division, heterodyne, and time expansion (for reviews see of Parsons et al., 2000; Parsons and Obrist, 2004. The Ultrabat records ultrasound directly and a custom-designed conversion program (datTOwav) converts the binary data files to wav files. These wav files were analysed in time expansion on a personal computer (Dell Inspiron 2200 using the software Batsound (v3.31 Pettersson Elektronik AB, Uppsala) with a sampling frequency of 31270Hz, 16 bits/ sample, and a 512pt. FFT with a Hamming window for analysis.

For each social call recorded, the following parameters were measured: starting frequency (Fstart), the frequency value measured at the beginning of the call; maximum frequency (Fmax), the highest frequency value observed within the call; end frequency (Fend), the frequency value measured at the end of the call; minimum frequency (Fmin), the lowest frequency value observed within the call; frequency of maximum energy (Fmaxe); total call duration (Dur); and inter-call interval (ICI, the time interval between two consecutive social calls). Figure 2.2 shows a sonogram of two typical *P. auritus* social calls with the various call parameters highlighted. ICI and D were measured from oscillograms, Fmaxe (kHz) from power spectra, and all other spectral parameters (kHz) from spectrograms. The time between consecutive social calls was measured in milliseconds. If the time between social calls was longer than two minutes, then the time was estimated to the nearest second. If more than one harmonic was present, values of the first (fundamental) harmonic were taken. Incomplete records and those with poor resolution were excluded from the analysis.

Calls that were clearly identified as *P. auritus* echolocation calls (as shown in Figure 2.3) were excluded from the analysis. *P. auritus* are sometimes referred to as whispering bats due to the quietness of their echolocation calls, which are emitted through their mouth or nostrils (Schober and Grimmberger, 1997). The echolocation call of *P. auritus* was analysed by Ahlén (1981) who described it as a faint and short FM sweep, about 2 ms long with prominent second harmonics, and a fast pulse rate (Russ, 1999).

2.2.3 – Principal Component Analysis

Patterns in data can be hard to find in multi-dimensional and large datasets (such as the call database described in this chapter). Principal Component Analysis (PCA) is a powerful tool for identification of patterns in data and to present the data in such a way as to highlight the similarities and differences. PCA begins with a matrix that represents the relationship between variables. The mathematical technique used in PCA is called eigen analysis and the eigenvalues and eigenvectors of the matrix are calculated using the sums of squares and cross products (Jolliffe, 1986). The eigenvector associated with the largest eigenvalue has the same direction as the first principal component. The eigenvector associated with the second largest eigenvalue determines the direction of the second principal component. The sum of the eigenvalues equals the trace of the square matrix and the maximum number of eigenvectors equals the number of rows (or columns) of the matrix (Jolliffe, 1986). Therefore, the first principal component is the linear combination that explains the most variance and the last principal component is the remaining combination of features that explains the least variance in the data (Wold et al., 1987).

The six acoustic parameters (Duration, Fmin, Fmax, Fstart, Fend, Fmaxe) were subjected to a PCA in order to explore the variance within the dataset. Not all components are retained in the analysis, and there is debate over the criteria used to decide whether a factor is statistically important. Only components with large eigenvalues should be retained, but deciding what constitutes an eigenvalue large enough to represent a meaningful component is more problematic. One method devised to decide the number of components is to plot a graph of each eigenvalue against the component with which it is associated. This graph is known as a scree plot and the cut off point for selecting components should be at the point of inflexion of the curve (Cattell, 1966). However, Kaiser (1960) recommended retaining all components with eigenvalues greater than one. This is based on the premise that eigenvalues represent the amount of variation explained by a principal component and that an eigenvalue of one represents a considerable amount of variation (Kaiser, 1960). With these considerations in mind, a scree plot was constructed to help establish the number of components to be retained, in addition to Kaiser's (1960) recommendation of retaining only principal components with an eigenvalue greater than one.

2.2.4 – Cluster Analysis

The objective of cluster analysis is to separate a set of objects into constituent groups (classes, clumps, clusters) so that members of any one group share similar properties and differ from one another as little as possible, according to a chosen criterion (Späth, 1980). The ultimate objective is to minimise within-group variance and maximise between-group variance. Almost all clustering techniques involve a process of measurement, either of the magnitude of distance between two objects, or of the magnitude of their similarity to each other, where objects are described by the values of the variables in the data matrix. The

choice of measurement used will influence the shape of the clusters, as some elements may be close to one another according to one distance and farther away according to another (Romesburg, 2004). The most straightforward and most common technique of calculating distances between clusters in a multi-dimensional space is to calculate Euclidean distances. However, the Euclidean distances can be greatly affected by differences in scale among the dimensions from which the distances are calculated and, therefore, transforming the dimensions to ensure that there are similar scales is an important step.

Once the distances between objects have been determined, the next step in the clustering process is to create the clusters. There are a number of different linkage and amalgamation rules that exist to help determine when two clusters are sufficiently similar to be linked together. For example, single linkage determines the distances between two clusters by the distance of the two closest objects (i.e., by the nearest neighbours) whereas complete linkage determines the distance between clusters by the greatest distance between any two objects in the different clusters (i.e., by the furthest neighbours). The choice of linkage or amalgamation rule depends in part on whether the objects actually form naturally distinct clumps or whether the objects are elongated 'chain like' clusters (Hartigan, 1975).

Traditional clustering methods fall into two broad categories: partitioning and hierarchical. Partitioning methods (such as k-means) move records iteratively from one cluster to another, starting from an initial partition. However, the number of clusters must be specified in advance, which is arbitrary, and this does not change during the iteration. Furthermore, k-means, for example, does not handle outliers very well (Jain and Dubes, 1988). Hierarchical clustering is advantageous in that, unlike partition methods, you don't need to specify the number of clusters in advance. However, it is difficult to process with large datasets, as it is

slow and problematic when the data contains a high level of error. Therefore, both of these traditional clustering methods were considered unsuitable for the initial cluster analysis because (i) the number of the clusters could not be identified objectively and (ii) the database of social calls was very large. More recently probability models have been proposed (Banfield and Raftery, 1993; Dasgupta and Raftery, 1998; Fraley and Raftery, 1998) using the Bayesian information criterion to compare multiple models and identify the optimum number of clusters. The model based cluster analysis approach has been shown to perform statistically better than the traditional methods (Fraley and Raftery, 1998) and also provides a measure of the uncertainty of the resulting classification.

With these considerations taken into account, cluster analysis using the model-based approach (Fraley and Raftery, 1998) was conducted on the call database (data matrix) for whereby the calls are classified on the values of the variables, which are the measured acoustic parameters (Duration, Fmin, Fmax, Fstart, Fend, Fmaxe). The resulting factor scores from the PCA (with an eigenvalue greater than one) were also subjected to a cluster analysis. The clustering technique used was MCLUST extension to R statistical software (Fraley and Raftery, 2006). The cluster model and number of clusters were further verified by subjecting a random selection of 75% of call database to MCLUST a further five times, each time using a random selection. As an additional verification, a supervised clustering was also conducted, using a traditional partitioning method, k-means cluster analysis, whereby the analysis specified to split the calls into the number of clusters determined by the model based approach. This was then compared to the model based analysis in order to ascertain whether there was agreement between both methodologies.

2.2.5 – Analysis of call sequences using Markov chain analysis

In order to examine whether there was any evidence of sequences of calls, in the form of syntax (patterns in which call types from the resulting clusters are ordered and combined) a first order Markovian chain analysis was applied. A Markov chain is a process that consists of a finite number of states and some known probabilities p_{ij}, where p_{ij} is the probability of moving from state *j* to state *i* (Norris, 1998). The controlling factor in a Markov chain is the transition probability; it is a conditional probability for the system to go to a particular new state, given the current state of the system (Revuz, 1984). The computer programme generated a matrix of the probability that one call type immediately followed the same or a second call type for each call type in the data set.

2.2.6 - Statistical Analysis

The descriptive analysis (mean, standard deviation, standard error, variance) and K-means clustering was carried out using SPSS (version 17.0 for Windows) for all of the 11,464 call parameters measured. A custom-designed MATLAB program, written by Lucas Wilkins at the University of Sussex, performed the PCA and MCCLUST Version 3 for *R* (Fraley and Raftery, 2006) carried out the model based cluster analysis. The ellipses for the clusters were drawn using a custom designed programme in Phyton v2.6 for Windows. The analysis of call sequences (Markov chains) were conducted using a custom designed programme in Phython v2.6 for Windows.

2.3 - Results

A total of 11,484 *P. auritus* social calls were recorded at the 20 maternity roost sites from May 2007 to September 2007.

2.3.1 - Descriptive variability of P. auritus *social calls at maternity roost sites.*

Analysis of ultrasound social calls (n= 11,484) showed that the majority of calls (n = 11,101) were comprised of a downward FM sweep, descending in frequency from a mean of approximately 52kHz to about 14kHz. A sonogram of a typical *P. auritus* 'Type A' call is shown in Figure 2.4. There was, however, a lot of variation within the acoustic parameters measured, detailed in Table 2.1 and Figure 2.5, specifically variations in Fmax(e).

The second most frequent call type (n = 215) recorded, which differed in spectrogram structure to Type A calls, was characterised by an upward sweep followed immediately by a falling FM sweep, as shown in Figure 2.6. This type of call was also described for *P. auritus* recorded at swarming sites by Furmankiewicz (2004). These calls, termed 'Type B', could be differentiated objectively from Type A calls on the basis of their starting frequency being lower than the maximum frequency within the call, as shown in Figure 2.7. The variations within the acoustic parameters measured are detailed in Table 2.2 and Figure 2.8

The final call type (n = 168) that could be distinguished objectively based on call structure, was characterised by a downward FM sweep followed by an undulating FM sweep, as shown in Figure 2.9. This type of call was also recorded by Furmankiewicz (2004) at *P. auritus* swarming sites and was described as 'v calls'. These calls, termed here 'Type C', could be differentiated from Type A and Type B calls on the basis of their end frequency being higher

than the minimum frequency within the call, as shown in Figure 2.10. The variations within the acoustic parameters measured are detailed in Table 2.3 and Figure 2.11.

2.3.2 - Relationship between acoustic parameters of the calls

The relationships between the acoustic parameters of each call were analysed and the results of the Spearman's rank correlation are detailed in Table 2.4. There was a significant negative correlation between the duration of a *P. auritus* social call and both the minimum and end frequency of the call, as shown in Figure 2.12 (a) and Figure 2.12 (b), respectively. Calls that were longer in duration tended to have lower minimum and end frequencies than shorter duration calls. There was also a significant negative correlation between the duration of a *P. auritus* social call and the peak frequency of maximum energy, as shown in Figure 2.12(c). The minimum frequency was positively correlated with peak frequency of maximum energy, as shown in Figure 2.12(d).

2.3.3 – Principal Component Analysis

The results of the PCA analysis, shown in Table 2.5, show that the first principal component accounted for 51.49% of the variance in the data matrix and the second principal component accounted for 31.82% of the variance in the data matrix. The scree plot, shown in Figure 2.13, plots the eigenvalues of the components in descending magnitude. The point of inflexion on the curve showed that there was no single clear elbow. However, only two components had eigenvalues greater than one, therefore, only these were retained.

A 2D loading plot was constructed, shown in Figure 2.14, to examine how the variables load onto each factor. Fmax and Fstart are heavily loaded on the first axis. This is unsurprising as these high frequency call parameters are likely to be extremely variable as a result of the attenuation of calls at high frequencies. The second component is more interesting, with three large positive loadings, Fmin, Fend and Fmax(e) and one large negative loading, duration. This further demonstrates the relationship between call duration and frequencies (described in Section 2.3.2), whereby longer social calls are characterised by low end and minimum frequencies and low frequency of maximum energy. A 3D histogram of the first two components was plotted against frequency. The resulting histogram, shown in Figure 2.15, demonstrates that the social calls tend to overlap in the parameters measured, but that there is some evidence of clusters of calls that have similar distributions in acoustic parameters.

2.3.4 – Model-based cluster analysis

The majority of calls (96.66%) were classified as 'Type A' calls, on the basis of sonogram structure. Although Type A vocalisations shared the same basic pattern, it was a very large group within which there was a lot of variation in acoustic parameters. In order to examine whether Type A calls could be further separated on the basis of the measurements of their acoustic parameters, the call database of Type A calls was initially subjected to a cluster analysis using the acoustic variables measured. The default settings for the EMclust command in MCLUST tested from 1 to 20 clusters and 10 models. The models tested are described further in Fraley and Raftery (2006). The resulting Bayesian information criterion (BIC) was used to determine which was the best model and number of clusters. This initial exploratory analysis revealed that the best model was 'EEV', a model with an ellipsoidal distribution, equal volume, equal shape, and variable orientation, as shown in Figure 2.16.

However, the model appears to over fit the data (which may be as a result of random error or noise) reaching asymptote initially at six clusters but increasing again at nine and 16 clusters. There are also missing values, and this suggests that one or more of the estimated covariance matrices are judged to be too close to singularity (Fraley and Raftery, 1998).

The factor scores from the PCA, with an eigenvalue greater than one, were subsequently subjected to the cluster analysis in MCLUST (as described above) in order to ascertain whether reducing the number of factors resulted in improving the fit of the statistical model. The resulting BIC, shown in Figure 2.17, indicated that the model reached asymptote at four (or arguably five) clusters but began to decrease after six clusters. Subjecting five random selections of 75% of the acoustic parameter data revealed that the EEV model was consistently the best model to fit the dataset, but the number of clusters as having the highest BIC value did change, as shown in Table 2.6. Six clusters, however, did end up in the top three BIC values on all five runs (whereas four and five did not). Therefore, the EEV model with six clusters was chosen as the most conclusive option to describe the call distributions of the data set.

The ellipses representing the six Gaussian clusters fitted to the data distribution are shown in Figure 2.18. The measurements of the acoustic parameters from the call, with the greatest probability of belonging to each of the cluster, are detailed in Table 2.7. Calls in overlapping clusters one and six were characterised by a duration of 9.8 ms and 11.1ms, a minimum frequency of 10.6 kHz and 13.2 kHz, and a frequency of maximum energy of 21.8 kHz and 19.6 kHz respectively. Calls in cluster two were longer in duration (12.4 ms), had similar minimum frequency to cluster one (10.6 kHz), and a lower frequency of maximum energy (14.5 kHz). Calls in clusters three and four were characterised by having a duration of 9.0 ms

and 10.9 ms, a higher minimum frequency (15.0 kHz and 17.3 kHz), and higher frequency of maximum energy (27.2 kHz and 31.2 kHz), but differed from one another in their starting frequencies, which were in the region of 43.7 kHz for cluster three and 52.5 kHz for cluster four. Calls in cluster five were characterised by having duration of 12.3 ms, a minimum frequency of 13.2 kHz, and a frequency of maximum energy at about 19.60 kHz. The clusters were not discrete and overlapped in their distributions. Therefore, there was the potential for some calls to be misclassified into the incorrect cluster. The probability values for the classification of each cluster with another cluster type are shown in Table 2.8.

2.3.5 – K-means cluster analysis

The acoustic parameters of the 11101 Type A calls were used as input into a k-means cluster analysis to determine homogeneous groups. Based on the Bayesian information criterion (section 2.3.5) the analysis aimed to separate the calls into six clusters where each of the calls was allocated to the cluster with the nearest mean and the results are shown in Table 2.9. The Euclidean distances between the final cluster centres are detailed in Table 2.10. The analysis showed that there was, in general, good agreement between the two clustering methods. Clusters two, three and five had a similar number of calls, with similar acoustic properties for both clustering methods. Cluster one differed between the two methods primarly in the acoustic parameter of the minimum frequency which was 10.6 kHz for the model based analysis and 13.4 kHz for the k-means analysis. There were also more calls in cluster one using the k-means (n = 2590) compared to the model based analysis (n = 2084). Cluster four has less calls using the k-means approach (n = 1525) compared to the model based approach (n = 2411) and were shorter in duration (8.72 ms and 10.9 ms respectively). Cluster six had

more calls using the k-means appraoch (n = 2566) compared to the model based appraoch (n = 1972) but, in general, the calls had similar acoustic properties using both methods.

2.3.6 – Sequence analysis – Markov chain

Only the calls from Type A were included in the Markov chain analysis (due to the low frequency of occurrence of Type B and Type C). The probabilities generated from the Markovian chain analysis, shown in Table 2.11, give a preliminary indication of the sequential predictability of call types. As shown in Figure 2.19, calls in cluster one, for example, has a probability of 48% for a call in cluster one to occur immediately after. This is then followed by a call typical of cluster six (19%), a call typical of cluster two (12%) and a call typical of cluster four (11%). Calls typical of cluster three and five have a 1.3% and 6.3% chance of occurring immediately after a call typical of cluster one respectively.

Each of the six call types classified was more likely to be followed by a repeat of the same call type than by any other call type, as shown in Figure 2.19. Call type 1 was first in the sequence (i.e. followed a period of silence) more often than expected and call Type 6 was first less in the sequence more often than would be expected from their frequencies alone, χ^2 1123.85, d.f. 5, $p < 0.0001$; shown in Figure 2.20. The probabilities of a third call type occurring in a sequence, given that the other two have occurred was not analysed. However, it was considered likely that multiple bat vocalisations may have contributed to the sequences which may confound the results.

2.4 - Discussion

A fundamental step for understanding animal auditory communication systems in the wild is to identify the acoustic characteristics of a signal. Categorisations of animal acoustic signals, by humans, have traditionally been based on spectrographic representations of the acoustic signal (Thorpe, 1954). Studies investigating bat social call structure have described individual elements of the call through visual examination of the call sonogram (Fenton, 1976; Barclay et al., 1979; Barclay and Thomas, 1979; Pfalzer and Kusch, 2003; Furmankiewicz, 2004; Jahelkova et al., 2008). This study also described individual elements of the calls through visual examination of the call structure, and found three distinct groups of social calls which could be objectively defined on the basis of their sonogram properties. However, 96.66% of calls had a similar basic call structure, an FM sweep descending in frequency from (mean values 52kHz to 14kHz). This similar structure is in contrast to other species' social calls, in particular CF-FM bats, the moustached bat *Pteronotus parnellii* (Kanwal et al., 1994), and horsehoe bats *Rhinolophus* sp.(Matsumura, 1979; Andrews et al., 2006; Ma et al., 2006), whose communication calls have a more variable call structure, including calls that are unique to the repertoire of CF-FM bats and not found in other bat species with more general echolocation strategies (Ma et al., 2006). However, although Type A calls were broadly similar in structure, there was a lot of variation in the acoustic parameters measured.

This study describes social call structure in one context (i.e. social calls recorded maternity roost sites). A study examining *P. auritus* social call structure in a variety of contexts, three swarming sites (one city park and two abandoned mines) and a maternity colony situated in a church attic, recorded 870 calls that were classified on the basis of visual appearance into six different call types (Furmankiewicz, 2004). These included V – shaped signals, when two or

more bats chased each other, (similar to the 'Type C' calls described in this study), and undulating FM calls followed by a descending FM sweep (similar to the 'Type B' calls described in this study)'. Furmankiewicz (2004) also noted that *P. auritus* produced 'rhythmic and rapid sequences of two, three or more (up to tens of) single FM type calls one after the other'. The study concluded that there was more diverse vocalisations produced at swarming sites, and that these diverse vocalisations 'probably play a role during the mating seasons of this species during spring and autumn swarming' (Furmankiewicz 2004).

An alternative approach to studying acoustic characteristics of a signal is to extract features directly from the acoustic signal. These features typically represent well-defined properties of audio signals, such as the duration of the call and the frequency bandwidths of the call. The main advantage in using this method is that experimenter bias can be avoided, but it is dependent on the features used, which may not always be extracted correctly by the experimenter. Taking the Type A social calls, and subjecting them to a PCA and cluster analysis, revealed that these calls could be further divided into subgroups or clusters on the basis of their acoustic properties. The best number of clusters ranged from four to six, with six being the strongest overall candidate. Cluster one overlapped with both clusters five and six, indicating that these clusters shared similar acoustic properties. Social calls in clusters five and six are characterised by a long duration (12.3 and 11.1 ms), low end frequencies (12.0 and 13.2 kHz), similar frequencies of maximum energy (19.6 kHz) but differ predominately in their start frequencies (46.8 and 52.3 kHz). It may be that these calls should actually be considered part of the same cluster as high frequency components of the calls may have been missing, as they attenuate more rapidly as they travel through the air (Arch and Narins, 2008). Furthermore, as a result of the directionality of many bat calls, the angle between the bat's mouth and the microphone of the recording equipment will affect signal

characteristics (Waters, 2001). One further potential issue with the low frequency sounds recorded was that the frequency response curve of the microphone has a low frequncy roll-off and fails to record frequencies below about 10kHz (indicated in Appendix A.4.2.2). The Knowles microphones are a commonly used microphone in commercial bat detectors as they produce a reliable frequency response curved in frequencies over 20kHz (Lars Petterson pers.comm. The lack of sensitivity to frequencies below 10kHz can be seen in Figure A.4.2.2 in the abrupt cut off in the properties of calls below this frequency. A total of 202 social calls (approximately 1.76% of the call database) had minimum frequencies below 10kHz. This means that the data presented for the minimum frequency (Fmin) are unreliable and that statistics that rely on this value are uncertain.

There was, in general, good agreement between the acoustic parameters of the clusters with both the model-based cluster analysis and the k-means cluster analysis. This agreement between the two clustering methods suggests that different clusters represent different call types even though the misclassification errors as a result of overlap are high. Therefore, a total of at least eight different *P. auritus* social call types were identified at maternity roost sites (six from 'Type A' calls and one each from 'Type B' and 'Type C'). The calls from Type B and Type C were not subjected to cluster analysis due to the low sample size and infrequency of occurrence. These calls were only recorded later in the season at maternity roosts (see Chapter 3).

Cluster analysis has been used to classify dolphin *Tursiops truncastus* whistles (McCowan, 1995) and humpback whale *Megaptera novaeangliae* social sounds (Stimpert et al., 2011). Wood et al., (2005) also used a model-based cluster analysis approach to classify elephant *Loxodonta africana* rumbles, where information pertaining to which elephant produced the

rumble was not available. They found that measuring the physical properties of 663 elephant rumbles, and subjecting these to cluster analysis, revealed three different types of rumbles that differed by their acoustic parameters, and these rumbles types were significantly associated with different behaviours. In this study it was not possible to associate call type with specific behaviour, but all the calls were produced in the vicinity of the roost site, and many were produced in the presence of other colonies members, in particular during swarming behaviour at dawn (see Chapter 3).

A number of studies have described vocal syntax for birds (Podos et al., 1999; Clucas et al., 2004; Berwick et al., 2011) whereas, by comparison, singing behaviour and syntax are exceedingly rare in mammals (Bohn et al., 2009). Evidence of patterns in call sequences has been described for moustached bats *Pteronotus parnelli* (Kanwal et al., 1994), horseshoe bats *Rhinolophus ferrumequinum* (Ma et al., 2006), and Mexican free-tailed bats *Tadarida brasiliensis* (Bohn et al., 2008). However, these studies did not go beyond determining that the order of call elements was non-random. Markov chains have been used to analyse and describe vocal syntax in birds (Ficken et al., 1994) and cetaceans (McCowan, 1995). More recently, a study by Bohn et al. (2009) found evidence using Markov chain processes that male Brazilian free-tailed bat song *Tadarida brasiliensis* produce songs with the same four types of syllables and the same three types of phrases across two regions. In this study no evidence was found that *P. auritus* produce sequences or phrases of calls at maternity roosts sites, only that calls are produced in sequences of similar call types (i.e. a call from one cluster is more likely to be followed by a call from the same cluster rather than a call from a different cluster). However, the Markov chain analysis conducted in this study was limited to the analysis of couplets and it may be that higher order sequences do exist.

There is evidence that communication calls in animals provide sufficient information for recognition of individual identity (Reby et al., 1998; Clark et al., 2006; Sproul et al., 2006). Studies investigating the variation in echolocation calls have found evidence that echolocation calls may provide information about the caller's identity (Kazial and Masters, 2004; Kazial et al., 2008a; Kazial et al., 2008b; Yovel et al., 2009; Voigt-Heucke et al., 2010), but, this may be species specific as not all studies have demonstrated such individual specific signatures for echolocation calls (Siemers and Kerth, 2006). Social calls, by contrast, are deemed to have primarily a communicative function, are more structurally complex, and therefore offer the possibility to encode both individual and contextual information (Russ and Racey, 2007; Carter et al., 2008; Melendez and Feng, 2010). Although this study did not examine aspects of individual recognition, as it was not known who or how many bats were calling, the variation recorded in the Type A social calls may be more specifically related to individual calling parameters as opposed to specific call types. The calls were however, recorded from 20 different roost sites in three separate geographic areas, which allows for an assessment of whether all call types occurred at all of the roosts or whether they were specific to roost types. This will be examined further in Chapter 3.

2.5 – Summary

Chapter 2 aimed to describe the structure of *Plecotus auritus* social calls recorded at maternity roost sites in Sussex. A total of 11,484 social calls were recorded and referred to as Type A, B and C.

- Type A calls (approximately 96% of the calls recorded) shared the same basic pattern (a FM sweep descending in frequency from a mean of approximately 52kHz to about 14kHz) but within this call type there was a lot of variation and principal component analysis and model based cluster analysis identified six separate clusters.

- Type B calls differentiated objectively from Type A calls on the basis of their starting frequency being lower than the maximum frequency within the call.
- Type C calls could be differentiated from Type A and Type B calls on the basis of their end frequency being higher than the minimum frequency within the call.
- Markov chain analysis of Type A calls found that for each of the six Type A calls classified was more likely to be followed by a repeat of the same call type than by any other call type.

Figure 2.1: Ultrabat recording equipment. The numbered parts are as follows:

1. PC (Torch Mini 12v pc (ESaw Ltd., London)
2. Timer / Control Unit
3. Power Supply – 12V Battery shown
4. Microphone amplifier (Knowles EK23132)
5. Waterproof case

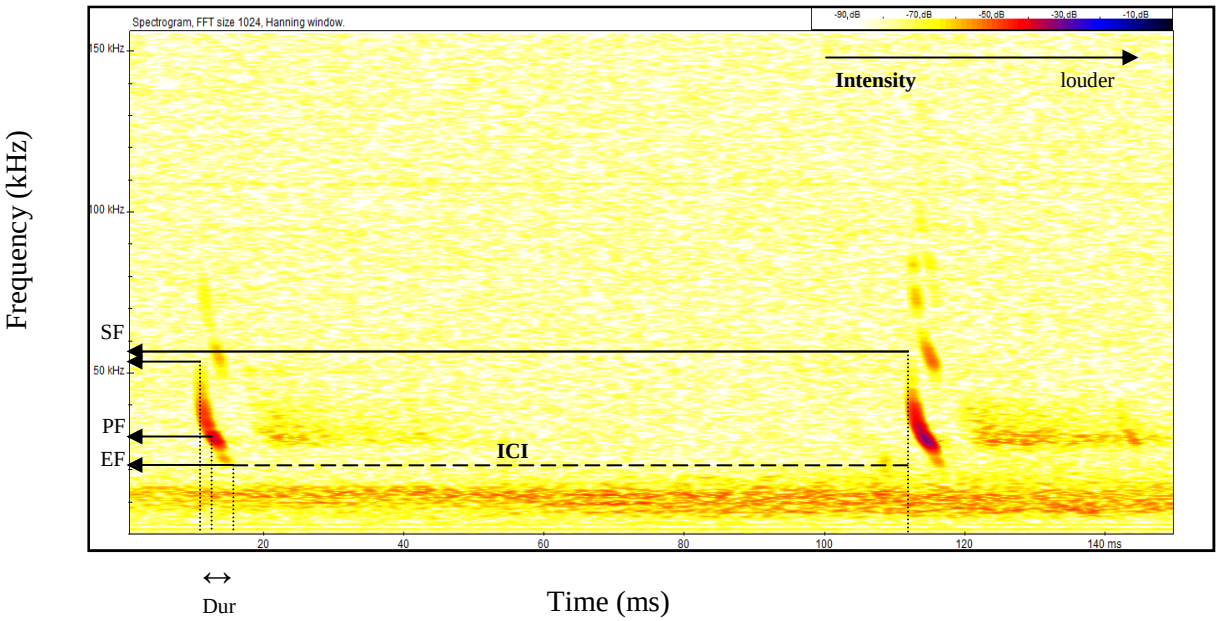

Figure 2.2: Sonograms of two typical Brown long-eared bat social calls. **SF** = the start frequency of the call, also here the maximum frequency (**FMax**), **PF** = peak frequency of maximum energy, **EF** = end frequency of the call, also here the minimum frequency (**FMin**), **Dur** = the duration of the call, **ICI** = inter-call interval between the end of one social call and the beginning of the next, Intensity = loudness of the call.

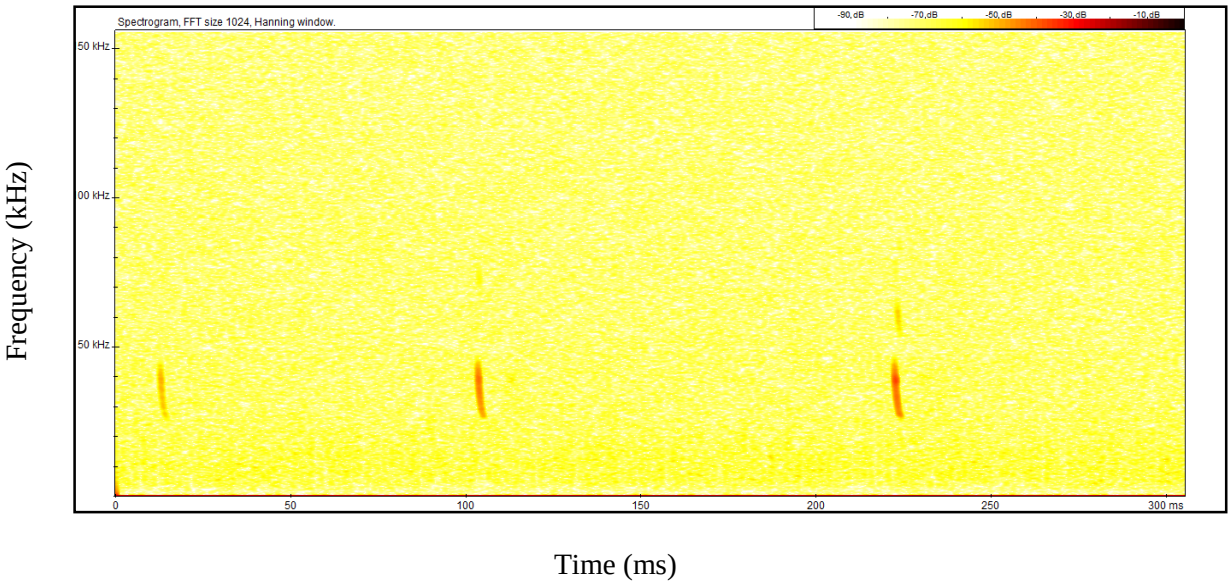

Figure 2.3: Sonograms of three pulses of typical Brown long-eared bat echolocation calls.

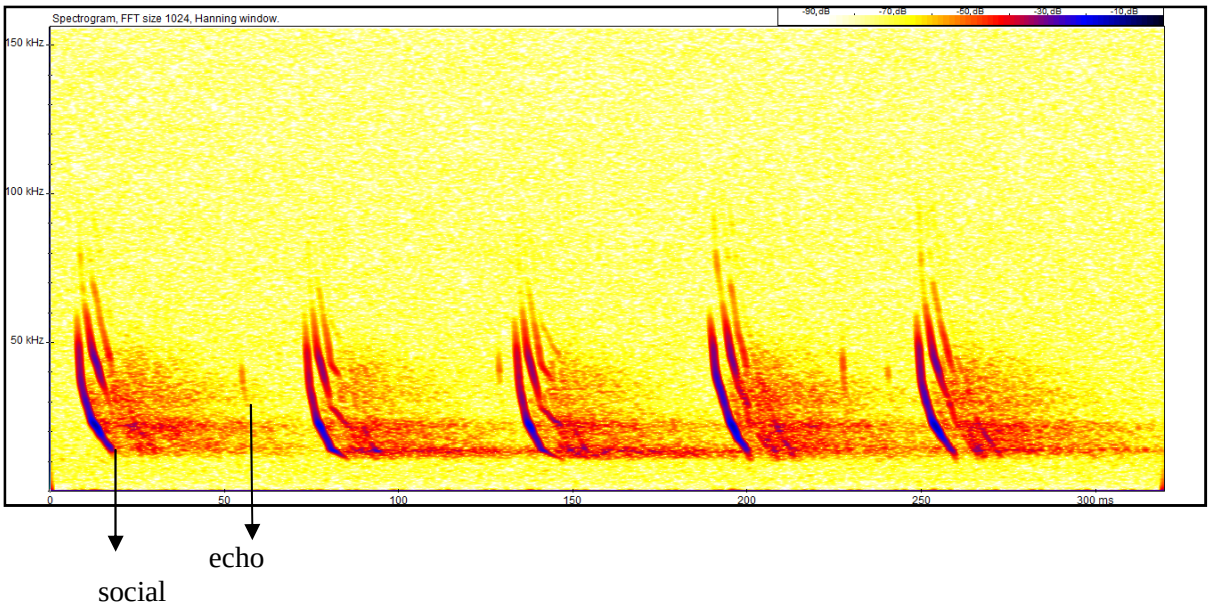

Figure 2.4: Sequence of *P. auritus* social calls, comprising of five social calls in the sequence. There is also clearly evidence of a second bat present as there is echolocation of *P. auritus* interspersed with the social calls.

	Mean	Minimum	Maximum	Std. Dev	Std. Error	Variance
Duration (ms)	10.61	1.43	19.80	2.11	0.02	4.43
Fmin (kHz)	13.95	8.00	41.20	3.81	0.04	14.58
Fmax (kHz)	51.97	19.60	73.20	4.87	0.05	23.78
Fstart (kHz)	51.97	19.60	73.20	3.81	0.05	23.78
Fend	13.95	8.00	41.20	3.81	0.04	14.58
Fmaxe	20.68	10.70	51.80	5.65	0.06	31.93
ICI (ms)	235972	0	19020000	1171940	11146	1.373E+12

Table 2.1: Descriptive statistics for the acoustic parameters of Type A social call (n = 11,101) recorded at maternity roosts.

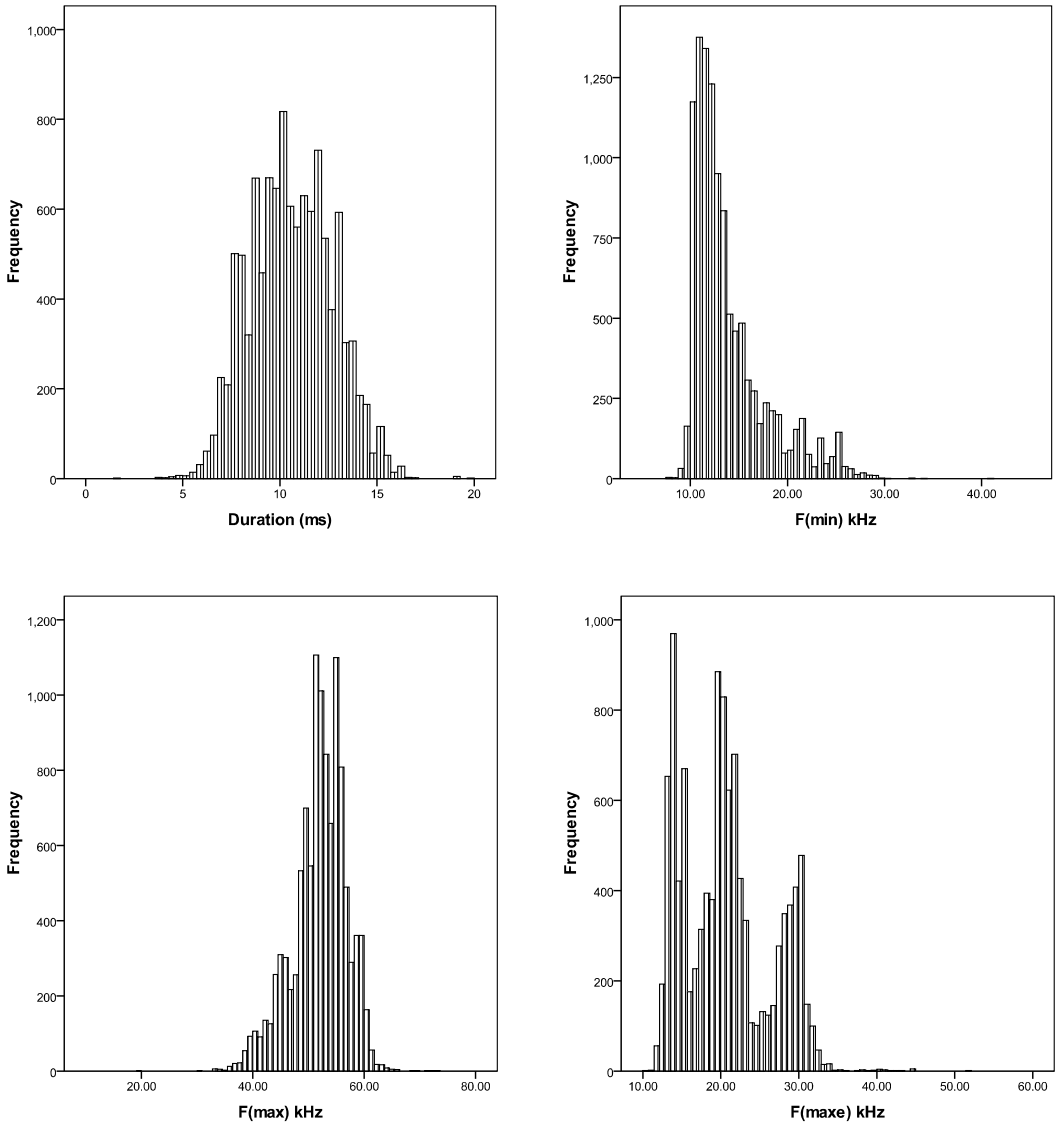

Figure 2.5: Variation in four acoustic parameters for Type A social call (n = 11,101) recorded at maternity roosts.

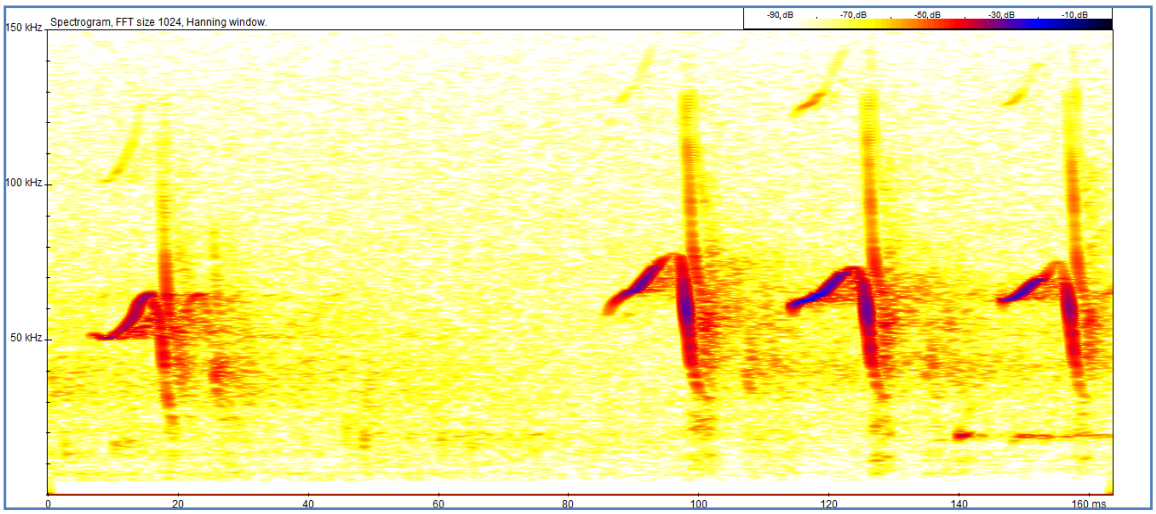

Figure 2.6: Series of four 'Type B' social calls, characterised by undulating upward FM sweep, followed by a descending FM sweep.

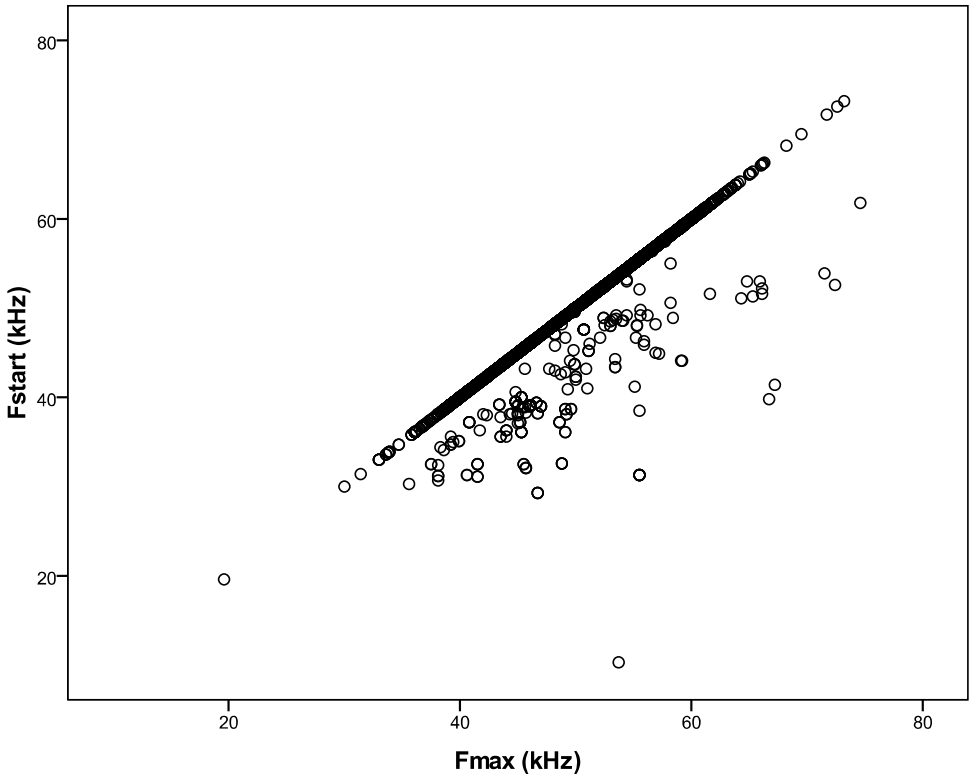

Figure 2.7: Correlations between the start frequency and maximum frequency of *P. auritus* social calls at maternity roost sites. The majority of calls had equal start and maximum frequency values, but Type B calls were characterised by a lower starting frequency value compared to the maximum frequency value recorded within the call structure.

	Mean	Minimum	Maximum	Std. Dev	Std. Error	Variance
Duration (ms)	10.01	5.20	22.90	2.60	0.18	6.76
Fmin (kHz)	17.45	10.10	37.20	5.08	0.35	25.85
Fmax (kHz)	49.15	35.60	74.60	6.84	0.47	46.84
Fstart (kHz)	41.24	10.30	61.80	7.20	0.49	51.87
Fend	17.45	10.30	37.20	5.08	0.35	25.85
Fmaxe	26.70	12.00	63.50	9.08	0.62	82.41
ICI (ms)	62546	0	9420000	9.17114E+5	62546	8.411E+11

Table 2.2: Descriptive statistics for the acoustic parameters of Type B social call (n = 215) recorded at maternity roosts.

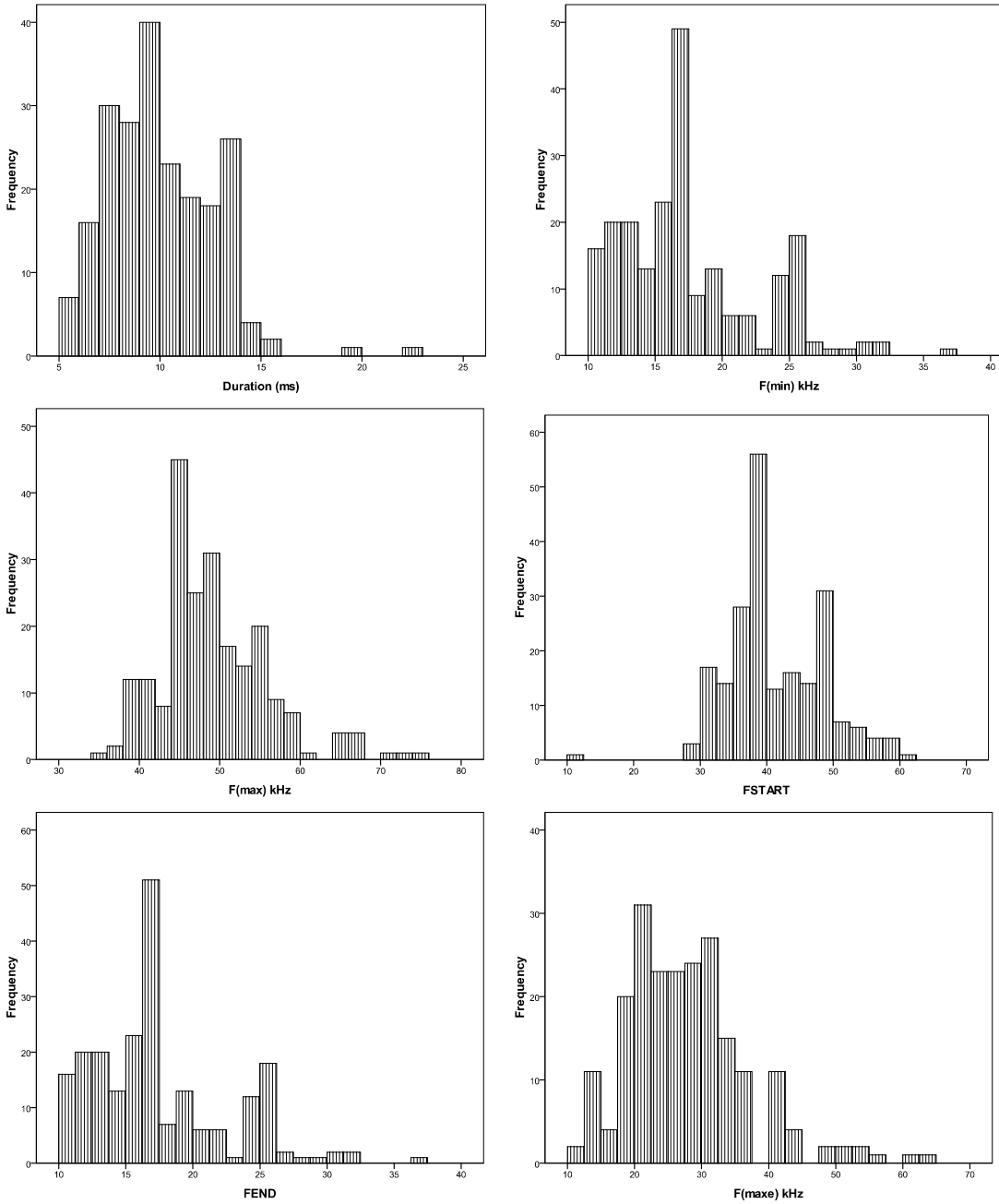

Figure 2.8: Variation in six acoustic parameters for Type B social calls recorded at maternity roosts (n = 215).

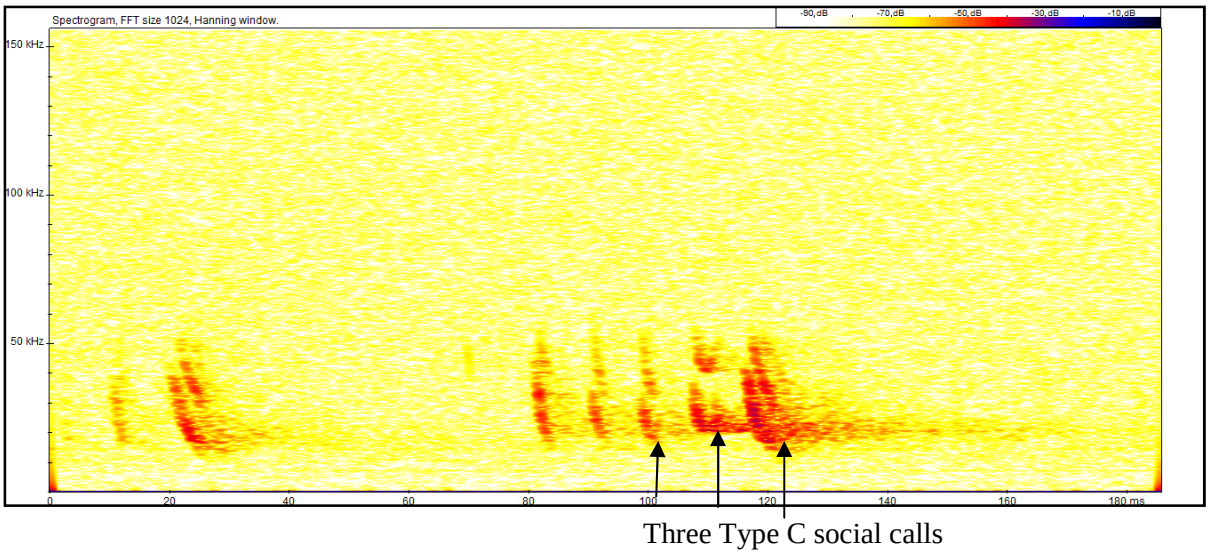

Figure 2.9: Series of seven social calls, three of which are classified as Type C calls, characterised by an upward FM sweep at the end of the call.

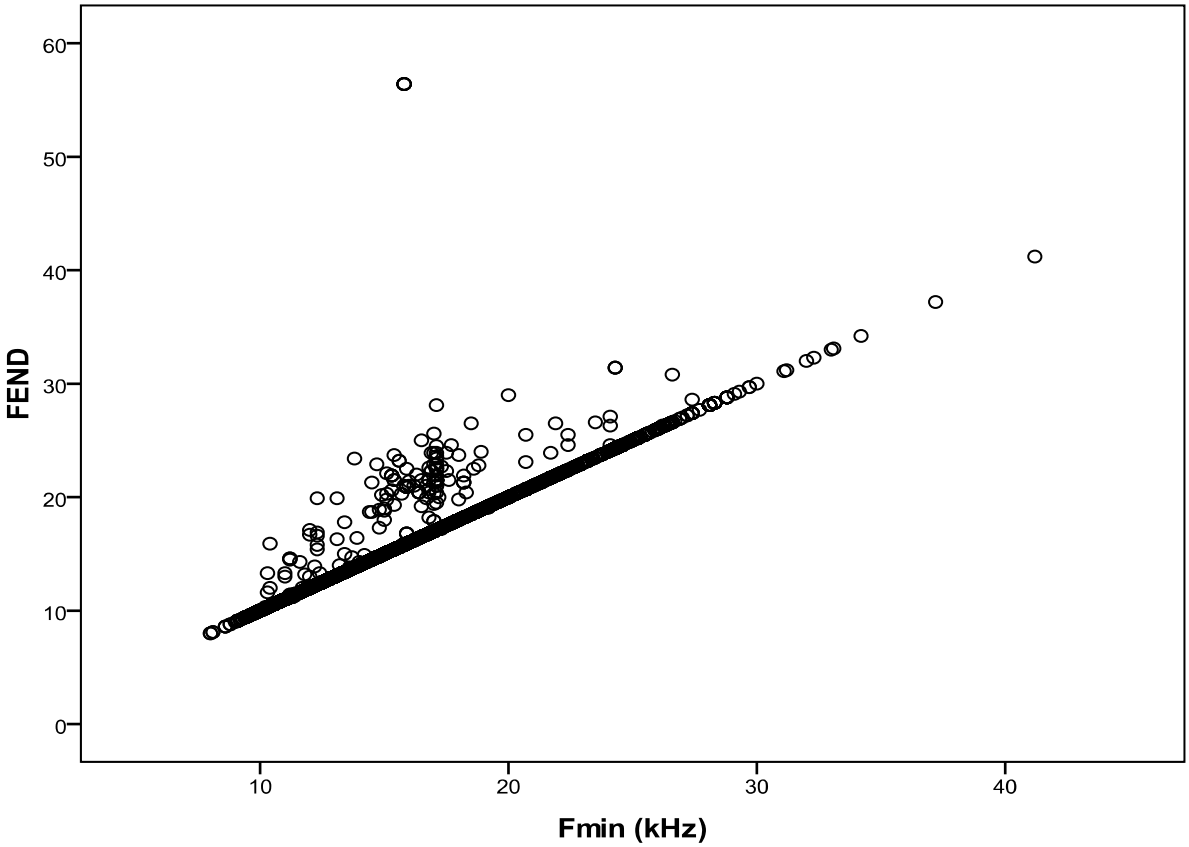

Figure 2.10: Correlation between the end frequency and minimum frequency of *P. auritus* social calls at maternity roosts. The majority of calls had equal end and minimum frequency values, but Type C calls were characterised by an end frequency that was higher than the minimum frequency.

	Mean	Minimum	Maximum	Std. Dev	Std. Error	Variance
Duration (ms)	9.29	4.10	13.3	1.75	0.14	3.06
Fmin (kHz)	16.27	10.30	27.40	3.13	0.24	3.13
Fmax (kHz)	49.87	31.40	59.40	6.11	0.47	37.28
Fstart (kHz)	49.87	31.40	59.40	6.11	0.47	37.28
Fend	20.55	11.40	31.40	4.04	0.31	16.29
Fmaxe	23.23	14.90	44.30	5.19	0.40	26.98
ICI (ms)	134833	0	7740000	8.11152E+5	62768	6.580E+11

Table 2.3: Descriptive statistics for acoustic parameters of Type C social calls (n = 168).

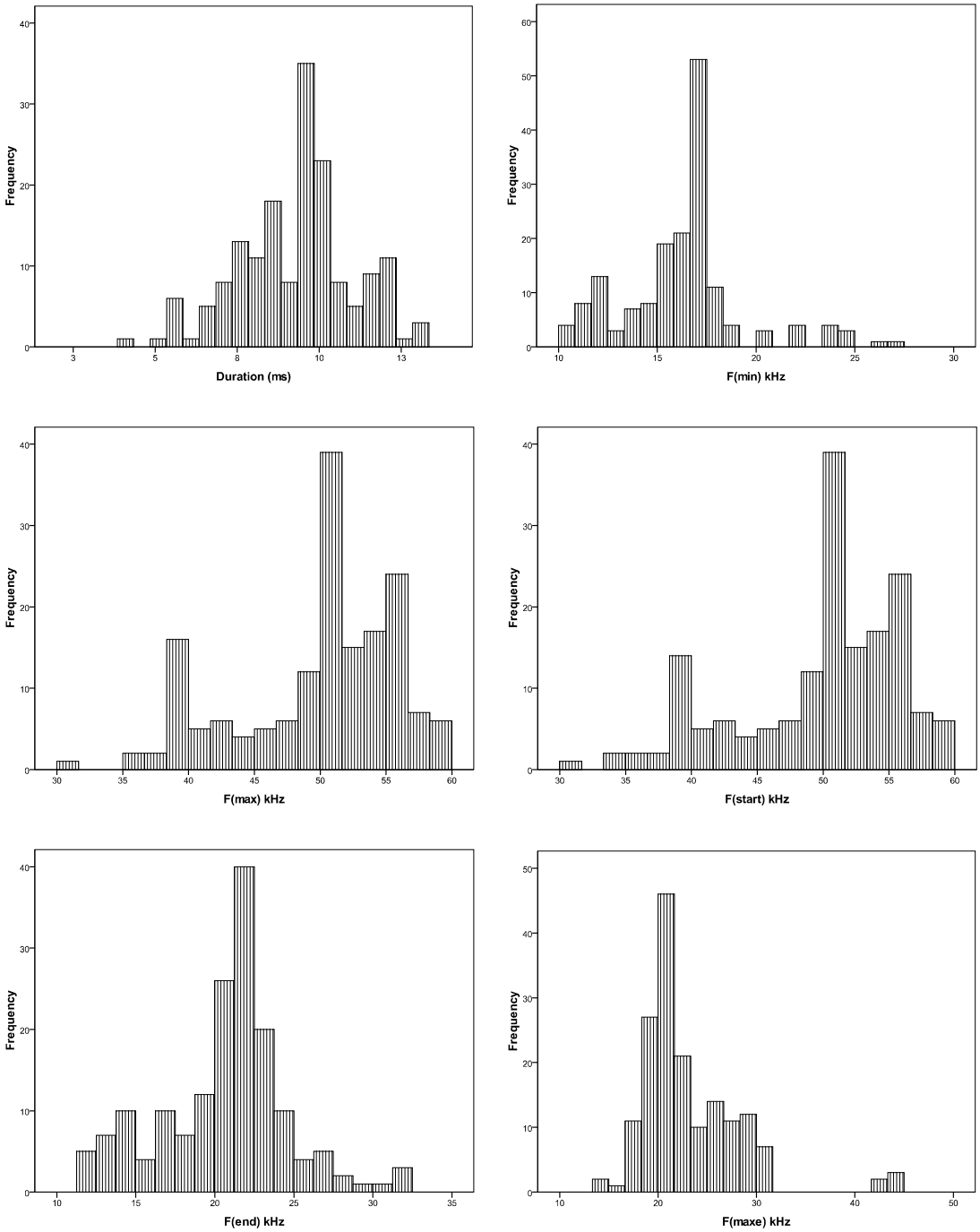

Figure 2.11: Variation in acoustic parameters for Type C social calls recorded at maternity roosts (n = 168).

	Duration (ms)	Fmin (kHz)	Fmax (kHz)	Fstart (kHz)	Fend (kHz)	Fmaxe (kHz)
Duration (ms)		r = -0.526*	r = 0.097	r = 0.099	r = -0.526*	r = 0.596*
Fmin (kHz)			r = -0.088	r = -0.099	r = 0.977*	r = 0.736*
Fmax (kHz)				r = 0.989*	r = 0.088	r = -0.043
Fstart (kHz)					r = -0.099	r = -0.055
Fend (kHz)						r = 0.737*
Fmaxe (kHz)						
	*Correlation is significant at 0.01 level (2-tailed)					

Table 2.4: Spearmans rank coefficient of correlation between six acoustic parameters of all social calls recorded at maternity roosts.

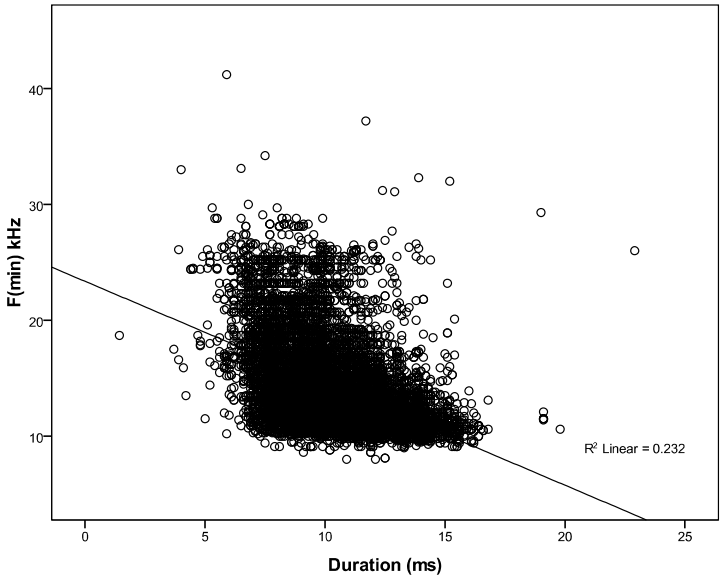

Figure 2.12(a): Correlation between duration and minimum frequency for all social calls at maternity roosts.

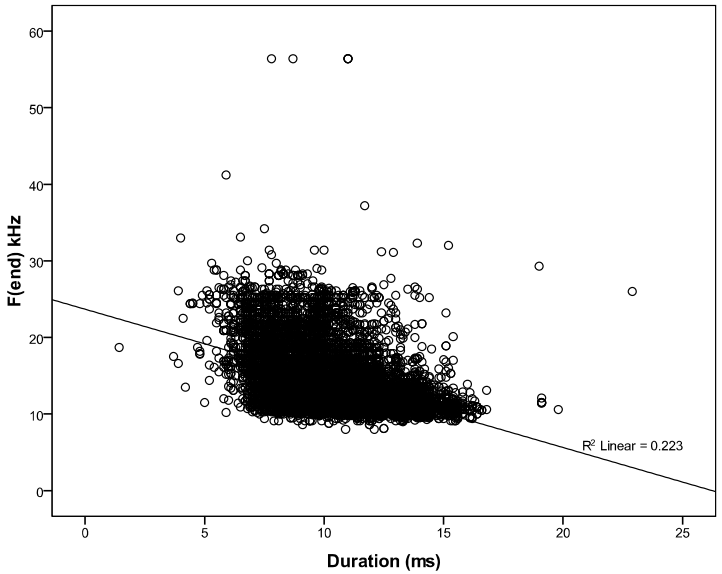

Figure 2.12(b): Correlation between duration and end frequency for all social calls at maternity roosts.

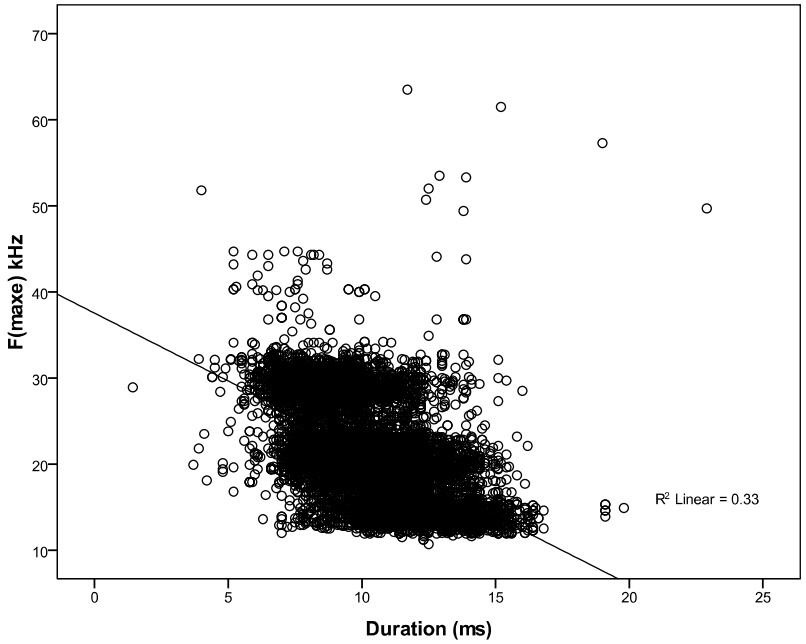

Figure 2.12(c): Correlation between duration and frequency of maximum energy for all social calls recorded at maternity roosts.

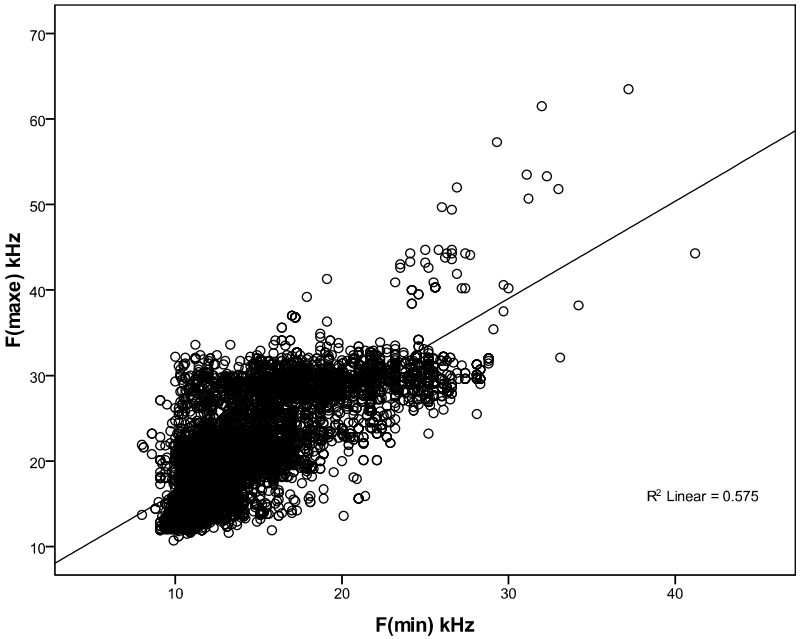

Figure 2.12(d): Correlation between minimum frequency and frequency of maximum energy for all social calls recorded at maternity roosts.

Component	Initial Eigenvalues			Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings[a]
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %	Total
1	3.089	51.490	51.490	3.089	51.490	51.490	3.051
2	1.909	31.819	83.309	1.909	31.819	83.309	2.003
3	.655	10.922	94.231				
4	.283	4.713	98.944				
5	.032	.538	99.483				
6	.031	.517	100.000				

Table 2.5: Results of the Principal Component analysis of 11,464 *P. auritus* social calls. The first two components account for approximately 83% of the variance in the data.

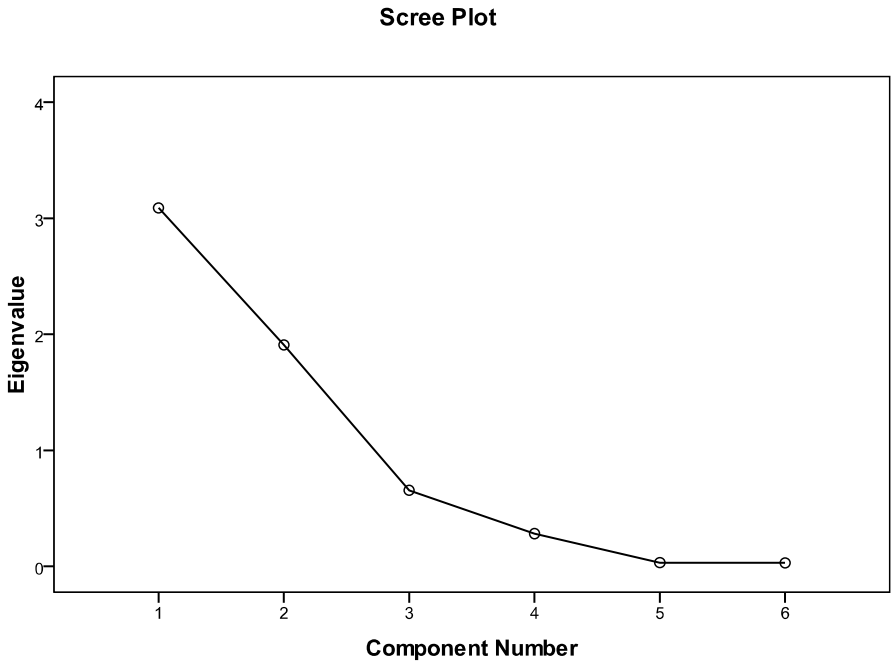

Figure 2.13: Scree plot of the eigenvalues from the Principal Component Analysis in descending order of magnitude. There is no clear single point of inflexion.

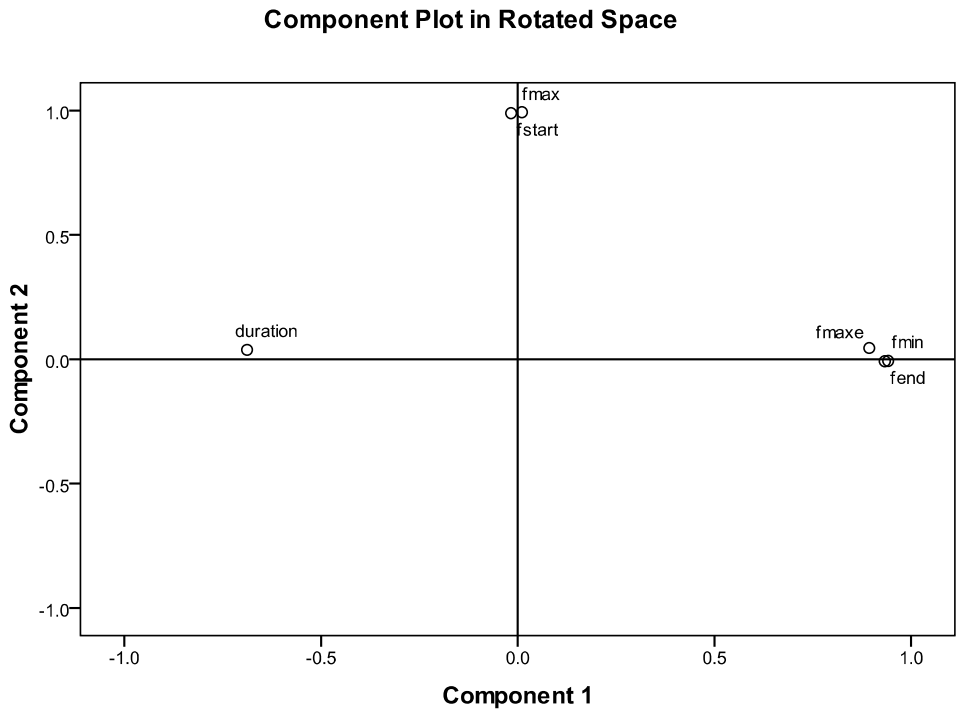

Figure 2.14: Location of the six acoustic parameters in relation to the two principle components identified by the analysis..

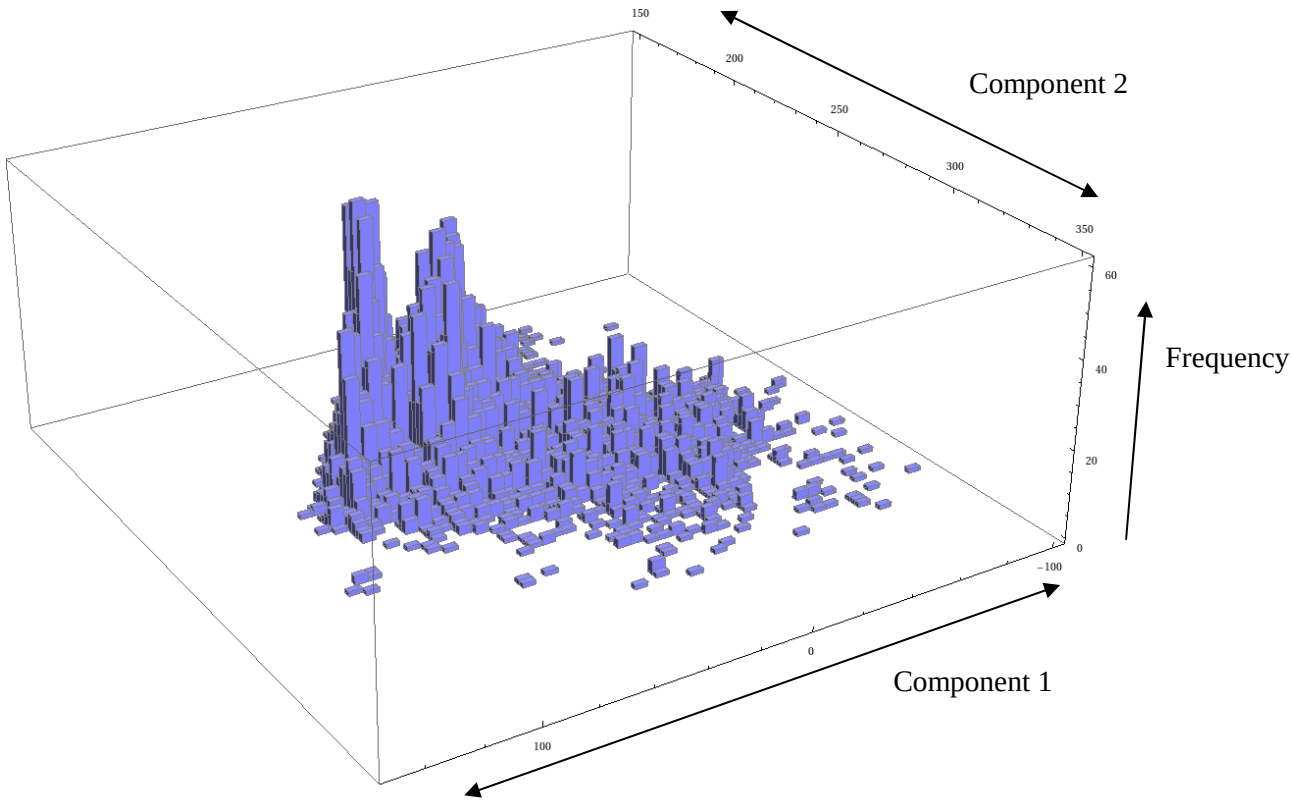

Figure 2.15: Frequency distribution of the first two components. The histogram demonstrates the overlap in parameters between calls.

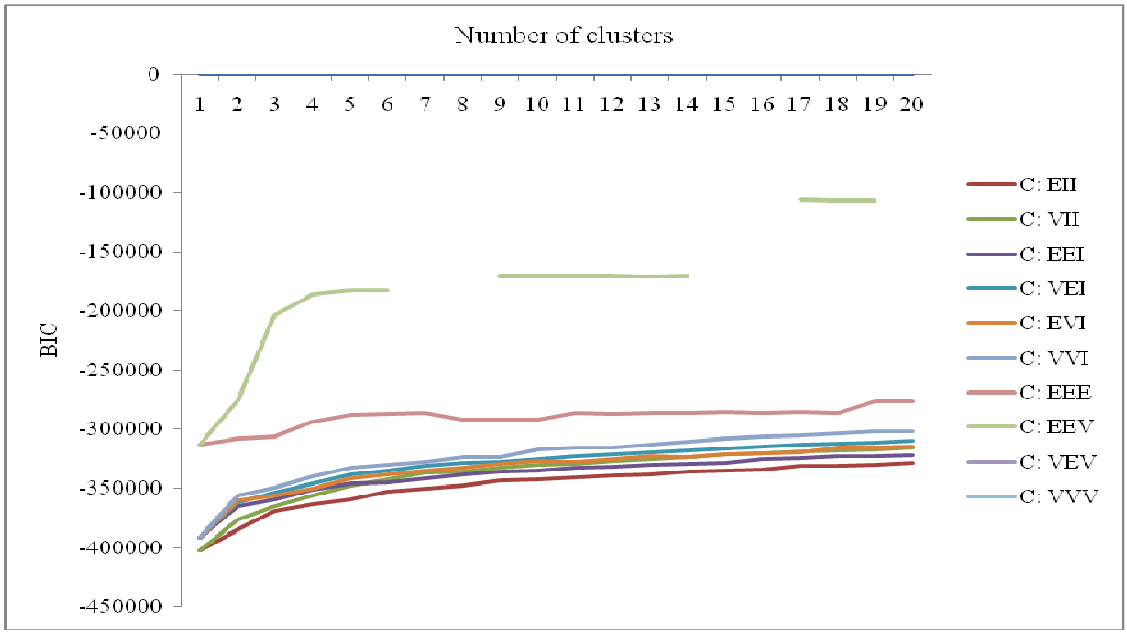

Figure 2.16: Bayesian information criterion values for 1 to 20 clusters for *P. auritus* call data. The 10 models tested (described in Farley and Raftery 2002) found that the EEV model was the best fit. However, missing values have occurred as a result of one or more estimated covariance matrices being too close to singularity (Fraley and Raftery, 1998) .

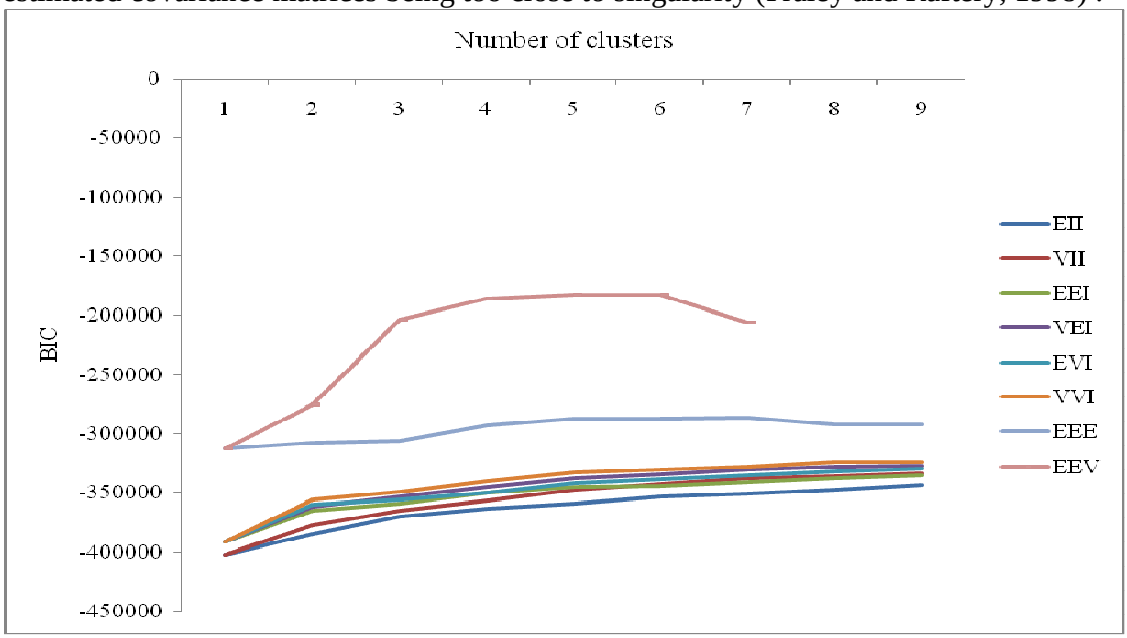

Figure 2.17: Bayesian information criterion values for 1 to 9 clusters for *P. auritus* PCA factor data. The 10 models tested (described in Farley and Raftery 2002) found that eight models fitted the data but the EEV model again was the best fit. The model reaches asymptote at four (or arguably five) clusters and begins to decrease after six clusters. Therefore, six clusters using the EEV model best fitted the dataset.

Run number	Number of clusters	Model	BIC value
1	6	EEV	-170220
	5	EEV	-170336
	4	EEV	-170556
2	9	EEE	-169987
	6	EEV	-170231
	5	EEV	-170344
3	6	EEV	-171211
	9	EEE	-171302
	4	EEV	-171399
4	7	EEV	-171399
	6	EEV	-171404
	4	EEV	-172509
5	5	EEV	-170356
	8	EEV	-170399
	6	EEV	-170443

Table 2.6: Results of the highest three Bayesian information criterion values for five runs of cluster analysis using a random sub-set of 75% of the acoustic parameters.

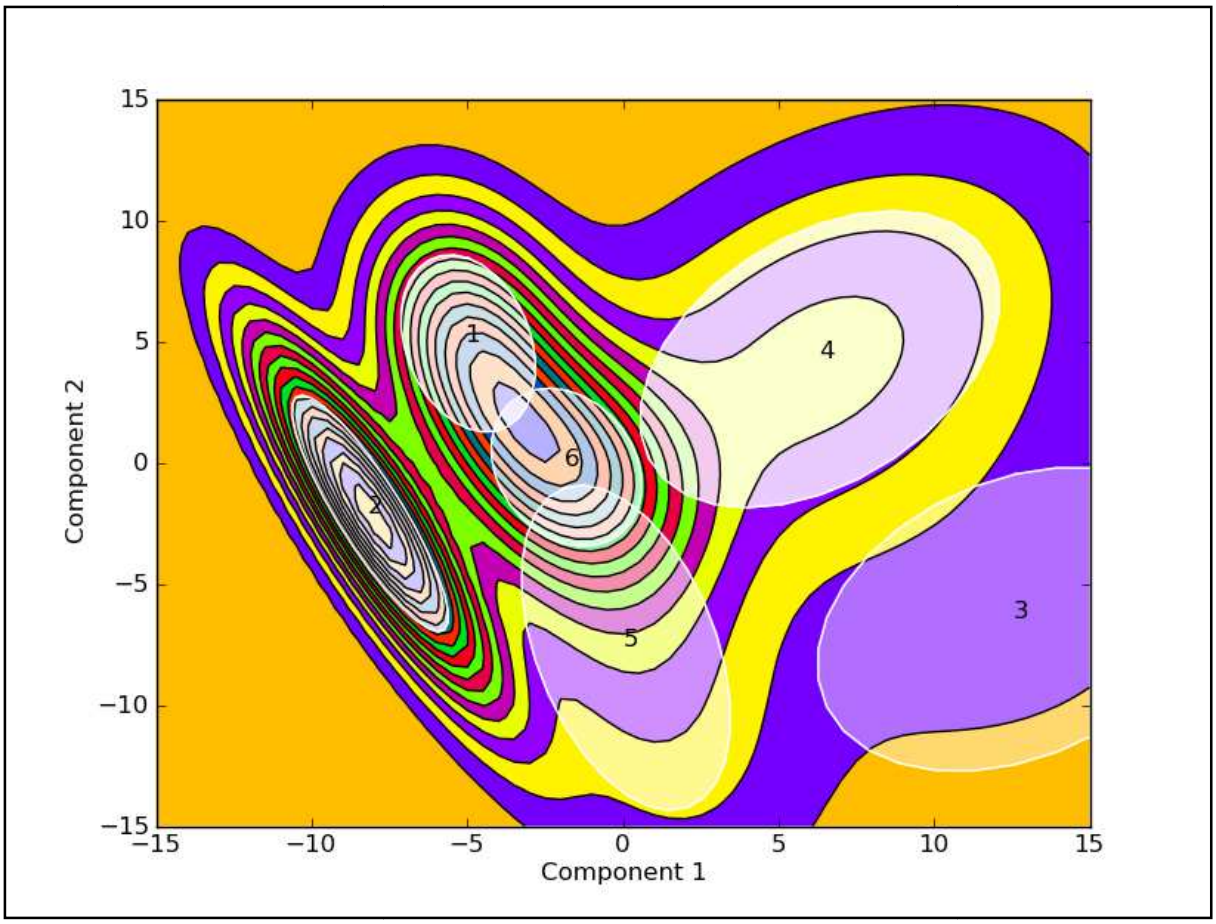

Figure 2:18: A representation of the six Gaussian clusters fitted to the data distribution. The contours represent frequency, whereby closely fitted contours indicate a high frequency of similar data points, such as the dense clusters one, two and six. Clusters three, four and five are more widely dispersed containing calls that are less similar to one another.

Cluster	Archetype call number	Duration	Fstart	Fmax	Fend	Fmin	Fmax(e)	No. of calls
1	832	9.8	56.3	56.3	56.3	10.6	21.8	2084
2	5470	12.4	53.1	53.1	53.1	10.6	14.5	2624
3	9640	9.0	43.7	43.7	43.7	15.0	31.2	842
4	10746	10.9	52.5	52.5	52.5	17.2	27.2	2411
5	4411	12.3	46.8	46.8	46.8	12.0	19.6	1168
6	10629	11.1	52.3	52.3	52.3	13.2	19.6	1972

Table 2.7: The acoustic parameters of the archetype social call with the highest probability of coming from each of the six clusters. The call number is the number of the call in the database. The number of calls is the total number of calls in each of the six clusters.

Clusters	1	2	3	4	5	6
1	0.746	0.018	0.001	0.048	0.035	0.152
2	0.029	0.889	0.001	0.021	0.041	0.188
3	0.000	0.001	0.849	0.113	0.036	0.000
4	0.010	0.000	0.064	0.850	0.028	0.047
5	0.003	0.047	0.108	0.100	0.658	0.089
6	0.144	0.007	0.004	0.149	0.151	0.542

Table 2.8: Probability values for the classification calls from one clusters with another cluster type. For example, there was a 2.9% chance of classifying a Cluster 2 calls as Cluster 1 and a 1.8% chance of classifying Cluster 1 call as Cluster 2.

Cluster	Number of calls	Duration	Fmin	Fend	Fmax	Fstart	Fmax(e)
1	2590	10.03	13.40	13.39	51.34	51.34	21.72
2	2400	12.29	11.20	11.20	52.06	52.06	14.47
3	778	8.88	16.06	16.06	45.43	45.43	28.51
4	1525	8.72	19.54	19.54	54.22	54.22	29.26
5	1242	10.83	12.38	12.38	43.58	43.58	18.55
6	2566	11.17	12.38	12.37	57.25	57.25	19.02

Table 2.9: K-means cluster analysis to partition the 11,101 Type A calls into six clusters in which each call belongs to the cluster with the nearest mean.

Cluster	1	2	3	4	5	6
1		21.18	19.50	15.22	12.65	12.28
2			12.86	19.34	16.15	11.55
3				8.87	22.17	8.27
4					22.93	8.97
5						15.33
6						

Table 2.10: K-means cluster analysis Euclidean distances between final cluster centres.

	No Call	1	2	3	4	5	6
No Call	0.000	0.290	0.210	0.080	0.240	0.080	0.100
1	0.010	0.481	0.127	0.013	0.113	0.063	0.193
2	0.012	0.109	0.633	0.010	0.089	0.058	0.089
3	0.013	0.038	0.031	0.476	0.268	0.119	0.054
4	0.006	0.090	0.080	0.080	0.598	0.038	0.107
5	0.009	0.081	0.146	0.094	0.111	0.408	0.151
6	0.005	0.196	0.131	0.023	0.137	0.098	0.410

Table 2.11: Probability values for the matrix of calls in each cluster type. The first column 'no call' represents silence. The values horizontally represent the preceding call and the values vertically represent the following call. As an example, there is a 12% chance of Call 2 following Call 1, and a 10% chance of Call 1 following Call 2.

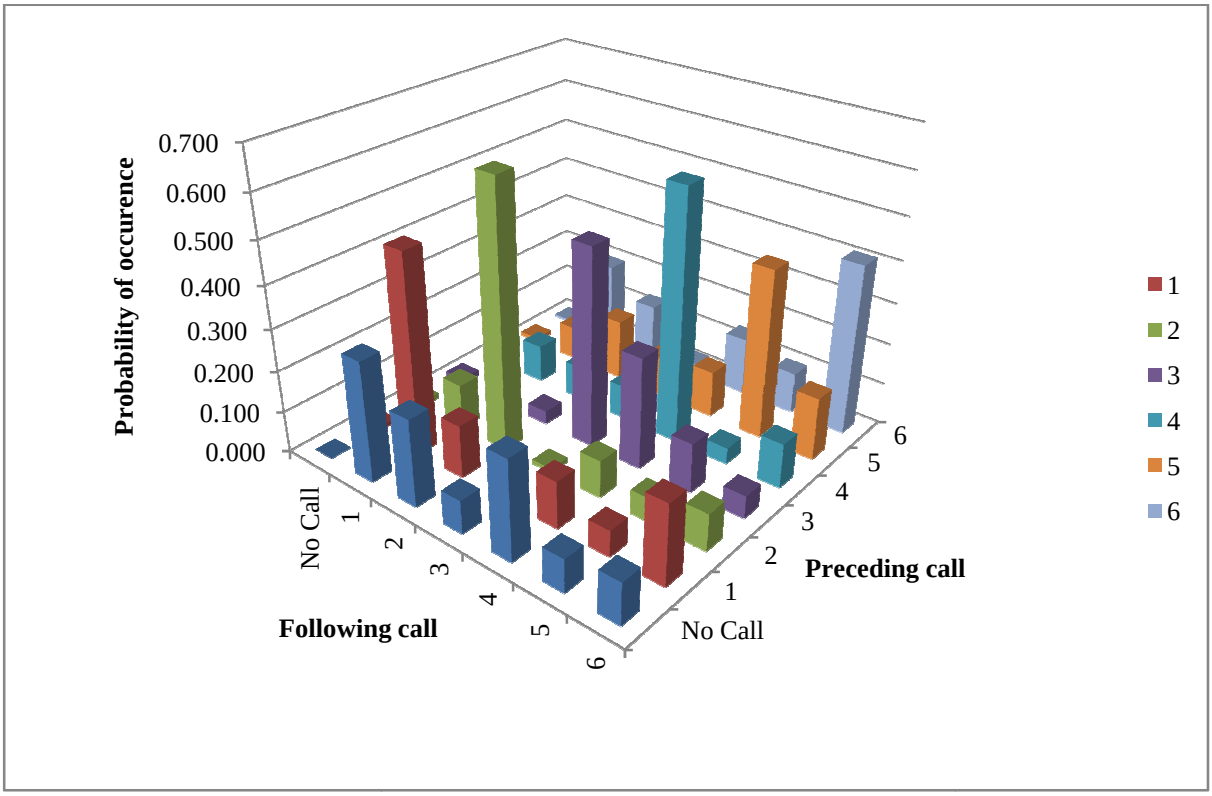

Figure 2.19: Three-dimensional bar chart of a transitional analysis consisting of pairs to show the probabilities with which one call type follows another.

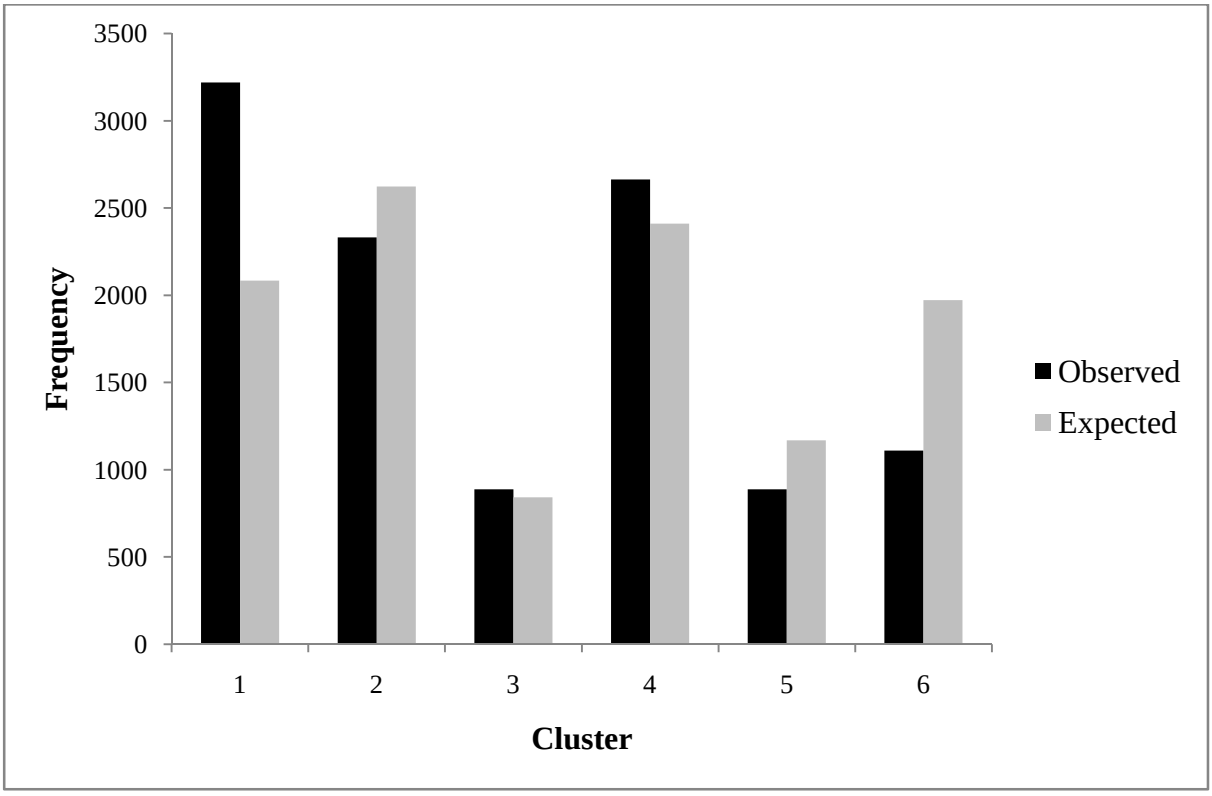

Figure 2.20: Observed and expected frequencies for each call type following silence. Calls type 1 was first more often than expected and Type 6 was first less often than would be expected from their frequency alone.

3.1 - Introduction

Bats occupying the temperate zone can be described as gregarious mammals that aggregate into groups for at least part of their lifecycle and occupy a variety of roost types in both man-made and natural structures. Benefits of group formation include, for example, reduced risk of predation (Parrish and Edelstein-Keshet, 1999) and, also, the reduction of individual energy expenditure in maintaining body temperature (Hayes et al., 1992; Berteaux et al., 1996; Gilbert et al., 2010). Roosts not only offer shelter and protection, but are also used for a variety of purposes including giving birth and raising offspring (Stebbings, 1966; Racey and Swift, 1985; Entwistle et al., 1997; Syme et al., 2001; Garroway and Broders, 2008), mating (Gerell and Lundberg, 1985; Kurta et al., 1993; Sachteleben and von Helversen, 2006) and hibernation (Kunz and Lumsden, 2003). The amount of time spent in roosts means that many social behaviours and interactions are likely to occur within the roost environment and roosts are also important to facilitate information transfer and social interactions within species (Wilkinson 1995; Kerth and Reckardt 2003).

Maternity roosts are usually occupied throughout the active season by females for the purpose of reproduction. Summer (maternity) roosts used by *P. auritus* are most commonly found in buildings, usually in the attic spaces of houses, barns or churches (Entwistle et al., 1997; Swift, 1998) but also in tree cavities (Horáček, 1975; Murphy et al., in prep) and in bat boxes (Boyd and Stebbings, 1989; Fluckiger and Beck, 1995). During the summer *P. auritus* forms stable colonies, comprised of both adult females and males and young of the year (Burland et al., 1999; Entwistle et al., 2000). *P. auritus* is rare amongst temperate zone bats in that adult males are regularly found with females in maternity colonies. Counts of bats

emerging from a maternity colony roosts are a common method of monitoring *P. auritus* populations (Bat Conservation Trust 2007).

Dawn swarming at the entrance to summer maternity roosts has been described for a number of species of bat (McAney and Fairley, 1988; Shiel and Fairley, 2000; Kanuch, 2007). Swarming behaviour occurs when bats return to the roost after foraging, either early on after their first flight or just before dawn when they enter the day roost. Bats fly in wide circles at the entrance to the roost, sometimes pursuing one another (Shiel and Fairley, 2000). Often they appear to land and take off again multiple times before entering the roost. Several hypotheses have been put forward for the function of this behaviour but none have been tested. Proposed functions include information transfer about good foraging areas that night (Wilkinson, 1995; Wilkinson and Boughman, 1998), advertisement of roosting locations (Chaverri et al., 2010; Schöner et al., 2010), novel roost sites (Kerth and Reckardt, 2003), or re-affirmation of social bonds between roost mates and anti-predatory behaviour (Wilkinson, 1995).

The role of social calls in actively maintaining associations among roost members has received little attention. If one of the functions of social calls produced at roost sites is to facilitate group cohesion then it would be expected that the number of calls recorded at roost sites would be positively correlated with colony size, as all bats would vocalise as part of the bonding process. A study on the relationship between social vocalisations and group size in Hawaiian humpback whale *Megaptera novaeangliae* found that the rate of vocalisation was positively correlated with group size (Silber, 1986). Conversely, if only one or a few ‘dominant’ individual called at roost sites, providing information about roosting sites or good foraging areas, then it would be expected that there would be no relationship between colony

size and the number of social calls recorded. For example, dominant green woodhoopoes *Phoeniculus purpureus* vocalise when they leave a group for a new foraging site, thus advertising their departure and attracting the attention of other group members to follow (Radford, 2004). Additionally, if social calls at roost sites function to reunite adult females with off-spring when adult females return to the roost after foraging, then seasonal and temporal patterns should be apparent around the birthing and rearing period. Therefore, I wanted to examine whether there are temporal and seasonal patterns of calls and sequences produced at *P. auritus* maternity roost sites.

3.2 - Methods

3.2.1 – Roost locations

Ten summer roost sites used by *P. auritus* were identified in and around the Plashett Wood area, north of Lewes, East Sussex TQ 546110 115660 from a radio-tracking study (see Chapter 5), and were confirmed to be occupied by maternity colonies by emergence surveys Some of the roosts had previously been surveyed as part of a DPhil study on the comparison of the roost ecology of *P. auritus* and Serotine *Eptesicus serotinus* bats by Jessa Battersby in 1996 (Battersby, 1999). The 10 roosts sites (locations shown in Appendix A.3.1) were situated in and around Plashett Wood (Area 1). The minimum distance between two maternity roost sites was 20 metres and the maximum distance between any two maternity roosts was 4km.

In order to increase the number of roosts, and the geographic area in which the study was conducted, two additional areas were chosen to survey, Cowfold and the surrounding area in

West Sussex and the Ashdown Forest and the surrounding area in East Sussex. These areas were chosen because *P. auritus* roosts were previously identified (Battersby, 1999) in close proximity to one another and these could be accessed within a reasonable amount of time from the University of Sussex campus at Falmer. The three areas chosen for the study are shown in Appendix A.3.2.

Ten roosts were initially chosen in each of the additional areas but upon further survey it was found that some of the roost sites no longer had bats present and it also became apparent that it would not be feasible to record 30 roosts once per month throughout the season and it was therefore decided that five roosts from Area 2 in Ashdown Forest, East Sussex (locations shown in Appendix A.3.3) and five roosts from Area 3, in Cowfold, West Sussex (locations shown in Appendix A.3.4), in addition to the ten roosts in Area 1 would be studied.

3.2.2 - Preliminary roost inspections

Preliminary inspections were carried out at each of the roost sites in late April and early May 2007. These included an internal inspection to (i) ascertain whether *P. auritus* bats were present and where in the property the bats were roosting (if more than one roof space was present) and to; (ii) assess the number of bats present, their roosting positions within the building, and whether the roost was still likely to be functioning as a maternity roost (based on the number of droppings present and anecdotal information from the roost owners) also; (iii) carry out a preliminary dusk and dawn survey to identify the primary exit and entrance locations and finally; (iv) determine a suitable position for the recording equipment to be

situated for subsequent emergence surveys to obtain recordings of vocalisations from bats leaving and returning to their roost site.

3.2.3 - Emergence counts

Emergence counts were carried out on a monthly basis at all of 20 of the roost sites. A range of volunteer surveyors assisted with surveys from June to September including members of the local bat group, undergraduate and master's students from the University of Sussex, and individuals training for their NE bat licences. Volunteers have been used to record bats in monitoring programmes such as NMBP and roost surveys of species such as lesser horseshoes (Warren and Witter, 2002). Volunteers are potentially a valuable scientific resource providing manpower to conduct extensive surveys. Some scientists have questioned the reliability of data collected by inexperienced people (Darwall and Dulvy, 1996). However, other studies have found few differences in the data collected by experienced scientists and project volunteers. Standardisation and validation of the results collected by volunteers, however, is recommended (Silvertown, 2009).

With these considerations in mind, I accompanied each volunteer surveyor on the initial roost count and gave a brief training session prior to carrying out the roost count. Depending on their experience (and whether or not they were familiar with *P. auritus* calls on a bat detector) a recording of *P. auritus* echolocation and social calls was played, along with recordings of other house roosting bats that may be present, such as *Pipistrellus pipistrellus, P. pygmaeus* and *E. serotinus*. A field guide (Jones and Walsh, 2001) for identification of

bats in flight using bat detectors and flight patterns was provided, in addition to a bat detector (Batbox II Heterodyne) and a standardised recording sheet (shown in Appendix A.3.5).

I accompanied each volunteer surveyor at each roost site for their first survey and conducted an inter-observer reliability test comparing the number of bats emerging in each ten minute interval as recorded by myself and the volunteer surveyor, to ensure that our records of the numbers and species emerging were consistent. To maintain consistency each roost was assigned to one or two surveyors who then carried out all the counts at their roosts on a monthly basis. I set up the ultrasound recording equipment (the Ultrabat, see Chapter 2 for a description) at each roost site during the daytime and programmed it to commence recording 15 minutes before sunset and stop recording 15 minutes after sunrise. The Ultrabat recording device was placed adjacent to the primary roost exit and entrance (ascertained by the preliminary roost inspections) and the microphone of the Ultrabat was mounted on a 2 metre pole to enable recordings to be made of bats entering and exiting the roost, as shown in Figure 3.1. On occasion when either the equipment did not record accurately or the bats emerged from another exit (so the exact number that emerged was not determined or the equipment was situated in the wrong position) the count was repeated on a subsequent evening. Counts commenced 15 minutes before sunset and bats were counted at one minute intervals until at least 1.5 hours after sunset or until 15 minutes after the last bat had emerged. The direction of flight and other behavioural characteristics were noted as well as meteorological data such as temperature and humidity (recorded using Oregon Scientific WMR80 full wireless weather station) at the beginning and the end of the emergence survey and the light levels were recorded at 10 minutes intervals using a light meter (EasyView 30, Extech Instruments).

3.2.4 - Data analysis

Data were entered in Excel spreadsheets and analysed using the statistical package SPSS 17.0 for Windows.

3.3 - Results

3.3.1 - Emergence counts

Counts were obtained throughout the season from 20 *P. auritus* maternity roosts and these counts were used as a basis to estimate mean colony size for each roost across the season, as shown in Figure 3.2. The overall mean colony size was 25.8 bats (n = 20, s.e.m = 3.45). The smallest maximum count was 12 bats (August count, roost number 26, Green Lane Farm, Area 1). The largest maximum count was 98 bats (July count, roost number 9, Lantern Cottage, Area 2). Mean colony size before the first juveniles flew, was obtained by taking the maximum count for each roost during May and June, which was 25.9 bats (range = 8 – 81, s.e.m = 4.29). This increased to a mean of 32.9 bats (range 12 – 98, s.e.m = 4.63) when the juveniles became volant, obtained by taking the maximum count for each roost during July and August. There was therefore a mean increase of 30.55% in colony size, in 2007.

3.3.2 - Emergence behaviour

For all 20 *P. auritus* roosts, the mean time for emergence of the first bat was 21.9 minutes after sunset (s.e.m = 0.76, n = 100, range = 9 - 36), and the mean median bat emergence was 34.5 minutes after sunset (s.e.m = 0.67, n = 100, range = 16 – 53). A typical emergence

pattern was for 1 to 3 bats to emerge approximately 15 minutes before the main emergence. Bats would subsequently emerge in groups of 2 – 5 and typically flew directly to cover, such as hedgerows or trees in the vicinity of the roost. Median emergence time was significantly correlated with the time of sunset ($r^2 = 0.97$, df = 98 $p < 0.0001$, Figure 3.3), whilst none of temperature ($r^2 = 0.02$, df = 98, $p = 0.089$), humidity ($r^2 = 0.002$, df = 98, $p = 0.891$) or light intensity (lux) ($r^2 = 0.012$, df = 98, $p = 0.250$) showed any significant correlation with median emergence time.

The mean duration of emergence was 34.9 minutes (s.e.m = 1.10, n = 100, range = 11 – 58. Duration of emergence was significantly correlated with the number of bats emerging from the roost ($r^2 = 0.602$, df = 98, Figure 3.4). The relationship between rainfall and emergence time could not be tested for statistically because on very rainy nights the survey was abandoned (to avoid potential damage to equipment), and persistent rainfall (i.e. for at least 50% of the survey period) only occurred on six of the 100 nights that the emergence surveys were carried out (to prevent excessive rain potentially damaging recording equipment). Bats emerged as normal on all six nights.

3.3.3 - Social calls recorded at roost sites: seasonal patterns.

A total of 11,484 *P. auritus* social calls were recorded at the 20 maternity roost sites from May 2007 to September 2007. There were significant differences between months in the mean number of calls recorded at maternity roost sites (Kruskal-Wallis ANOVA H = 23.52, d.f = 4, $p < 0.001$; as shown in Figure 3.5). A total of 10 post-hoc pair-wise comparisons was undertaken (using Mann-Whitney U with a Bonferroni correction to reduce Type 1 error rate (.05/5) resulting in a critical value for significance of 0.01) to investigate where the

differences were significant. Figure 3.5 details the results of the differences in the mean number of calls per month. There were significantly more social calls recorded at maternity roost sites later in the season (September) compared to earlier in the season (May to June).

One possibility is that the increased number of social calls recorded later in the season may be a result of the longer recording time (as a consequence of fewer daylight hours). For example, the recording time from sunset to sunrise for roost number six was 445 minutes in June compared to 702 minutes in September. Therefore, the mean number of social calls per hour was calculated for each roost and there were significant differences between months in the social call rate per hour (Kruskal-Wallis ANOVA H = 10.47, d.f = 4, p = 0.03; as shown in Figure 3.6). A total of 10 post-hoc pair-wise comparisons was undertaken (using Mann-Whitney U with a Bonferroni correction to reduce Type 1 error rate (.05/5) resulting in a critical value for significance of 0.01) to investigate where the differences were significant. Figure 3.6 details the results of the differences in the monthly mean number of calls per hour. There were significantly more social calls per hour recorded at maternity roost sites in September compared to June (but not May).

The number of social calls showed a linear increase from June to September, whereas, the number of bats emerging decreased sharply from August to September, as shown in Figure 3.7. A Scheirer-Ray-Hare test showed that there were significant differences in the number of social calls recorded at maternity roost sites in relation to month and the number of bats emerging but there was no evidence of an interaction between month and number of bats emerging (Table 3.1). Nonetheless, the mean overall number of social calls recorded at each

roost site from May to September 2007 was positively correlated with the mean colony size, (Spearman rank correlation r_s =0.932, n = 20, p < 0.01) as shown in Figure 3.8.

3.3.4 - Temporal patterns of social call production

To examine whether there were temporal differences in the production of social calls at *P. auritus* maternity roosts, the number of calls emitted per quarter of night was analysed. The total length of time was calculated from dusk to dawn, for each night's recording, and it was divided into quarters. Each social call was then allocated to a specific time quarter from Q1 to Q4 and the social call rate per hour was subsequently calculated for each quarter. There was evidence of a difference in the mean number of social calls per hour on a quarterly basis per night, but no evidence of monthly differences or evidence of an interaction between month and quarterly night period, as determined by the results of the Scheirer-Ray-Hare test shown in Table 3.2 and Figure 3.9. Furthermore, the seasonal increase in the calling rate occurs in Q4. A Kruskal-Wallis test also found that there were significant differences between the social calls per hour analysed in quarterly periods (Kruskal-Wallis ANOVA 163.50, df = 5, p < 0.001). Post-hoc Mann-Whitney U tests were subsequently applied to investigate where the differences in temporal calling were significant. There were significantly more social calls per hour recorded in Q4 than in Q1, Q2 and Q3, significantly more calls recorded per hour in Q1 than in Q2 and Q3, and no significant differences between the number of social calls recorded in Q2 and Q3 as shown in Figure 3.10.

3.3.5 - Seasonal patterning of specific call types

In Chapter 2 the 11,484 calls recorded were classified into three types Type A, B and C, based on their call structure. The majority of calls (96.6% n = 11,101) were classified as Type A calls, and the cluster analysis indicated that these could be further subdivided on the basis of their acoustic parameters into six different call types (Calls 1 to 6, Type B and C were renamed Call 7 and Call 8 respectively). Using the data from the k-means cluster analysis (see Chapter 2 section 2.3.6), a significant association was found between call type and the month in with the call was produced (χ^2 = 1615, d.f 5, p > 0.001). However, this association test combined all calls and all months and did not provide any information as to which calls, if any, were more frequently associated with a particular month. Therefore, post-hoc analysis was carried out using cross-tabulation and converting standard residuals (the difference between observed and expected values) to a 'Z' score. This, in turn, was compared to a critical value for alpha using a conservative critical value of Z = +/- 3.15, (corresponding to a p value of 0.0016, applying a Bonferroni correction of .05/30 for multiple comparisons). Table 3.3 shows the results of the cross-tabulation for the χ^2 test of association.

Six positive and four negative associations were significantly greater than the critical value of Z = +/- 3.15, p = 0.0013. Call 1 and Call 5 were significantly associated with the month of June, Call 3 and Call 4 were significantly associated with the month of May, Call 2 was significantly associated with the month of September, and Call 6 was significantly associated with the month of August. By contrast, Call 2 was less frequently associated with the month of May, Call 3 and Call 6 were less frequently associated with the month of September, and Call 4 was less frequently associated with the month of June. However, as there was an overall increase in the number of social calls recorded in August and September, the number

of calls assigned to each cluster type was analysed as a proportion of the overall call database, for each month, as shown in Figure 3.11. Most of the calls did not exhibit a great deal of seasonal variation. Call 2, which is differentiated from the other call types by having a longer duration and lower frequency of maximum energy (see Chapter 2 section 2.3.5), showed an increase in occurrence from May to July, a small decrease in August followed by a sharp increase in September. Call 3 occurred at a higher frequency in May compared to the rest of the season. Call 7 and 8 comprised a very small proportion overall, 1.87% (n = 215) and 1.46% (n = 168) respectively, of the total number of social calls recorded at roost sites, and were therefore, analysed separately. The number of calls assigned to each call type was analysed as a proportion of the overall call database for each month. There was a significant difference between months (Kruskal –Wallis Anova H = 16.176, d.f. = 4, p = 0.003) with the majority of calls for both calls types recorded more frequently later in the season, as shown in Figure 3.12.

3.3.6 - Temporal patterns of specific call types

In order to investigate whether the six different Type A calls were more frequently associated with occurrence at a given time of night (i.e. when bats are leaving and/or returning to the roost, or when females returned to feed their offspring), the analysis was carried out using cross-tabulation and converting standard residuals, the difference between observed and expected values, to a 'Z' score which, in turn, were compared to a critical value for alpha. Using a conservative critical value of Z = +/- 3.09 (corresponding to a p value of 0.002, derived from a Bonferroni correction of .05/24 for multiple comparisons), Table 3.4 shows the results of the cross-tabulation for the Chi Sq. test of association. There were no calls associated with occurrence for each quarter of a nights' recording. The proportions of calls

for each quarter, shown by the bar charts in Figure 3.13, also reflect that all of the call types were equally likely to occur in similar proportions for each quarter of a nights recording. There were also no significant differences between quarters for call 7 (Kruskal-Wallis Anoca H = 4.691. d.f. = 3, p = 0.196) and call 8 (Kruskal –Wallis Anova H = 6.646, d.f. = 3, p = 0.084).

3.3.7 - Testing for roost and location call types

The six Type A calls occurred at all roost sites (although in different proportions see Figure 3.14). Type B (call 7) was recorded at 18 roost locations and Type C calls (call 8) was recorded at 15 roost locations. This suggests that the call types are likely to be species-specific as opposed to roost or location specific. However, the potential for further analysis of within call type for both roost and geographic areas are discussed in section 3.4.

3.4 - Discussion

The *P. auritus* maternity roosts surveyed in this study varied in size from as few as 12 bats up to 98, and the number of social calls recorded at these roost sites was highly correlated with the numbers of bats present in the colony. This correlation suggests that most individuals at maternity roosts may contribute to the vocalisations as opposed to a subset of dominant individuals repeatedly calling. *P. auritus* live in stable colonies comprised of matrilineally related females (Burland et al., 1999; Veith et al., 2004) with little immigration or emigration (Entwistle, 1994; Entwistle et al., 2000) and are long-lived. It is possible, therefore, that one function of social calling at *P. auritus* maternity roosts is to maintain and strengthen social bonds amongst colony members.

Social calls at roosts occur in the dawn swarming period (Briggs and King, 1998) and this study found that there were significantly more *P. auritus* social calls in the last quarter of the night prior to re-entry to the roost. The function of dawn swarming is not known for certain and several hypotheses have been put forward but none have been tested. These hypotheses include, for example, information transfer about good foraging areas that night, teaching the young to fly, and anti-predatory behaviour. However, calls may also be given periodically at roosting sites to attract conspecifics or guide colony members to potential roosts. A study by Schöner et al., (2010) demonstrated that simulated social calls attracted bats to roost sites and the bats could discriminate between the synthesised social calls of conspecifics and the calls of other sympatric species. Schöner et al., (2010) played back simulated Bechstein's bat *Myotis bechsteinii* and simulated Natterer's bat *M. nattereri* in an area of deciduous forest in Germany where *M. bechsteinii, M. nattereri*, and *P. auritus* colonies were present in an area of approximately 0.5km^2. They placed the speakers of the Sussex Autobat (Hill and Greenaway, 2005) inside bat boxes (without blocking the entrances) and subsequently emitted simulated *M. bechsteinii* and *M. nattereri* socials calls monitoring the responses using a combination of infra-red video recording and passive integrated transponder (PIT) tags. *M. bechsteinii, M. nattereri* and *P. auritus* approached the experimental bat boxes significantly more often when bat social calls were played back compared to control nights without calls. *M. bechsteinii* and *M. nattereri* approached the speakers broadcasting the call significantly more often when the calls of conspecifics had been played compared of the respective other species. Approaching *P. auritus* did not discriminate between *M. bechsteinii* and *M. nattereri* (Schöner et al., 2010)

An alternative hypothesis for the function of calls at roost sites would be that the calls serve to advertise the new roosting locations to conspecifics. A study by Chaverri et al, (2010)

showed that Spix's disk-winged bats *Thyroptera tricolor*, which had been captured and subsequently released close to an unfamiliar roost in which a conspecific had been placed, were attracted to the roost by social calls of this conspecific. Female *P. auritus* have been shown to switch roosts during the summer, particularly females utilising tree roosts (Murphy et al., in prep), and social calls may function to advertise new roosting locations to colony members. However, female *P. auritus* roosting in buildings show less evidence of roost switching behaviour (Entwistle, 1994; Entwistle et al., 2000; Murphy et al., in prep) but they do regularly switch roosting positions within buildings (Battersby 1999).

Animals may learn to recognise individually distinct calls of group mates. One method for advertising group membership is to adopt a common vocalisation (Wilkinson, 1995; Boughman, 1997; Boughman and Wilkinson, 1998; Frommolt et al., 2003). The greater spear-nosed bat *Phyllostomus hastatus* emits audible screech calls when departing from roosts that attract group members to foraging sites (Boughman, 1997; Wilkinson and Boughman, 1998). Group members could discriminate between calls of their groups members and calls given by other bats that were not members of their group (Boughman and Wilkinson, 1998). Analysis of acoustic variables of the screech calls given by *P. hastaus* found group differences but not individual differences in call structure (Boughman and Wilkinson, 1998). This study did not find any evidence that *P. auritus* colonies used different call types that could allow for discrimination between members of adjacent colonies or populations as all the call types were recorded at each of the roosts but there may have been differences within call type.

The increase in the number of social calls in September, despite the decrease in mean colony size, suggest that either all remaining bats are calling more frequently or individuals in the

vicinity of the roost site may be vocalising repeatedly or potentially both scenarios could be a contributing factor. In September, maternity colonies begin to disband as bats disperse to transient roosts prior to hibernation (Heise and Schmidt, 1988). There is some evidence that mating may occur in maternity roosts in *P. auritus* (Swift, 1998) and, as the mating process begins in autumn, it could be that the increase in social calls could be as a function of the onset of the mating process. Furmankiewicz (2004) reported that only sequences of FM calls (typical of type A) were recorded when bats were emerging or entering the summer maternity roost site and that the calls differed from the social calls recorded at swarming sites, which were more diverse in structure. However, Furmankiewicz (2004) only recorded 118 social calls at one maternity roost site in September. This study recorded 3626 social calls across 20 maternity roosts in September and found a low number of calls (approximately 8.14%) that differed in structure to the six Type A calls. These calls were categorised as Call 7 (Type B Chapter 2) and Call 8 (Type C Chapter 2). These calls were similar in structure to the undulating (Call 7) and V call (Call 8) described by Furmankiewicz (2004) at underground swarming sites in Poland.

Swarming at hibernation sites has been reported for *P. auritus* in continental Europe (Veith et al., 2004; Furmankiewicz and Altringham, 2007) and these studies have implied that the function of swarming may be related to mating and gene flow. However, studies on swarming at hibernation sites in the U.K. have found less evidence for *P. auritus* swarming behaviour (Greenaway, F. pers.comm) compared to other species such as Natterer's *Myotis nattereri* and Daubenton's bat *Myotis daubentonii* (Parsons et al., 2003) and, also, that the majority of *P. auritus* recorded at these sites were juveniles (Greenaway, F. pers.comm). Entwistle (1994) concluded that the likely mating system in *P. auritus* is a random, promiscuous one in which swarming occurs and females mate with many males, including mating by males with

torpid females at hibernation sites and mating in early spring. It is, therefore, possible that the increase in social calling at maternity roost sites in the UK in September may function in the mating process for males to advertise their presence to females at maternity roosts. Call type two, which was longer in duration with a lower frequency of maximum energy, occurred more frequently in September as did calls seven and eight, which were more diverse in call structure compared to the typical Type A calls that were recorded throughout the season.

Acoustic variation has been shown to occur in animal vocalisations at many different levels not only amongst group members. Most of the studies on variation in animal vocalisations have been carried out on birds. For example, regional variation or dialects have been demonstrated in the vocalisations of Black capped chickadee *Poecile atricapillus*. Upon analysis of gargle types, 88.7% were found unique to individual populations rather than shared among or between populations (Miyasato and Baker, 1999). A study of regional variation of the social calls of Kuhl's Pipistrelle *Pipistrellus kuhlii* found evidence that the peak frequencies of two adjacent populations differed (Russo and Jones, 1999). However, a study on the regional variation of complex bat songs in male Brazilian free-tailed bats *Tararida brasiliensis* did not find evidence of variation of song types or diversity between differing locations (Bohn et al., 2009). This study found that all of call types occurred at most of the roosts and all of the regions, but this study did not analyse within each call type to see if there were differences in the acoustic parameters at a finer scale. For example, differences between peak frequencies or maximum frequencies between adjacent colonies and / or aeras. However, this type of study may be better conducted under more controlled conditions where the identity of the individual calling is known and more precise measurements of the acoustic parameters of the social calls can be made. Furthermore, calls from captive individuals from known colonies could be used to test hypotheses with respect to call function, as well as

whether roost mates can discriminate roost members from intruders. These types of questions could be addressed by using playback techniques in controlled conditions.

3.5 – Summary

- Mean colony size was positively correlated with the number of social calls recorded.
- There were significantly more social calls recorded at maternity roosts later in the season (September) compared to earlier in the season (May to June). This is in contrast to the number of bats recorded emerging at roost sites in September.
- Temporal analysis of call production indicated that there were significantly more calls recorded in the time period (quarter of night) prior to dawn.
- Most of the call types described (Chapter 2) did not exhibit a great deal of seasonal variation but Call 2 (see Chapter 2 section 2.3.5), which is differentiated from other call types by having a longer duration and lower frequency of maximum energy, exhibited a sharp increase in September. Call 3 occurred at a higher frequency in May compared to the rest of the season.
- There was no evidence of calls being associated with occurrence for a given time of night (i.e. departing and /or returning to roost sites).

Figure 3.1: Ultrabat recording equipment placed outside roost B2 Sleeches Farmhouse. Number one denotes the primary exit location (from under the lead flashing adjacent to the rear chimney stack). Number two denotes the silver microphone box mounted on a pole to record bat vocalisations as the bats enter and exit the roost. Number three denotes the recording equipment placed inside a waterproof casing.

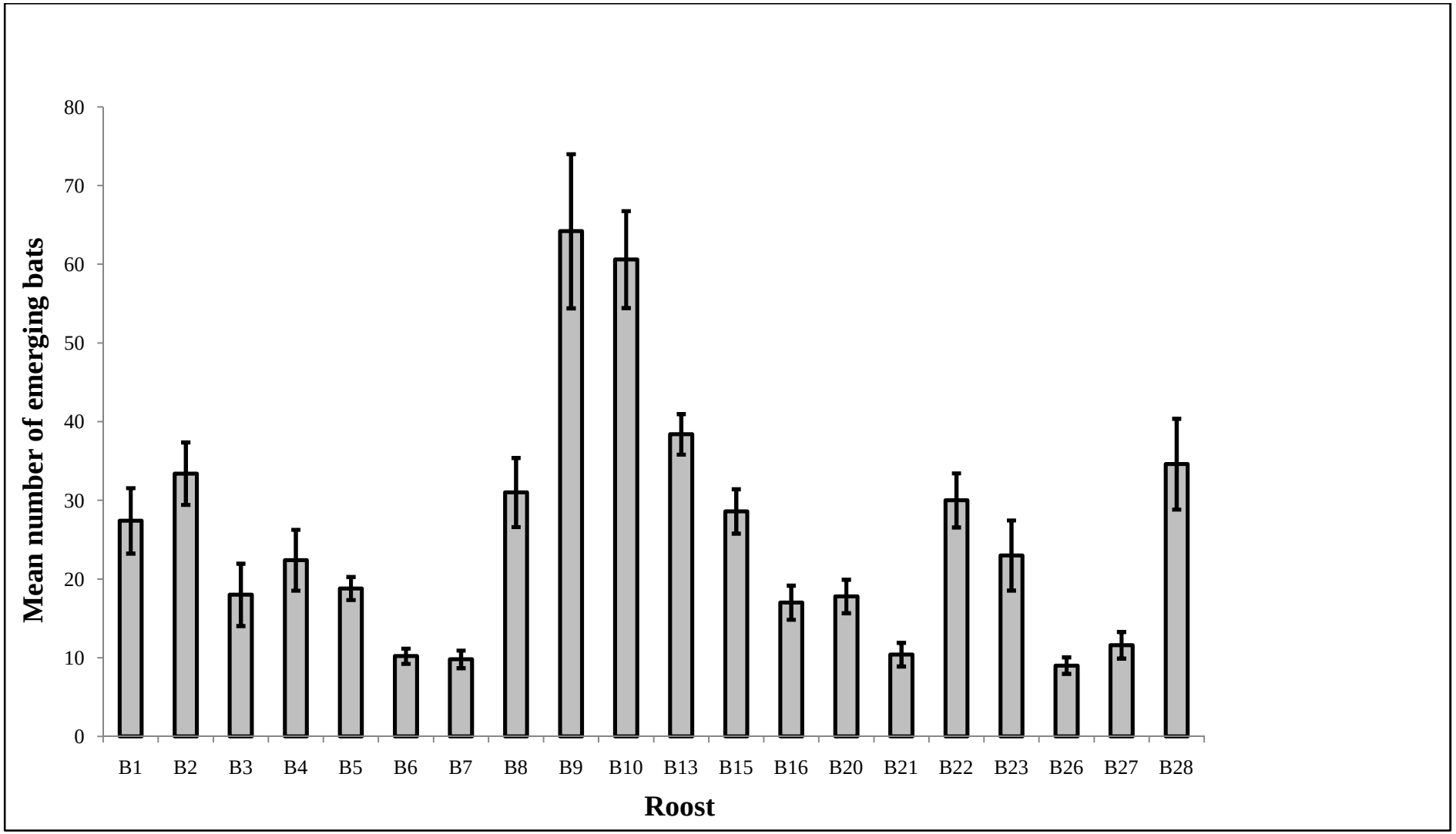

Figure 3.2: Mean emergence counts for each of the 20 *P. auritus* maternity roosts, obtained from monthly emergence survey counts, from May to September 2007. The error bars show the standard error of the mean.

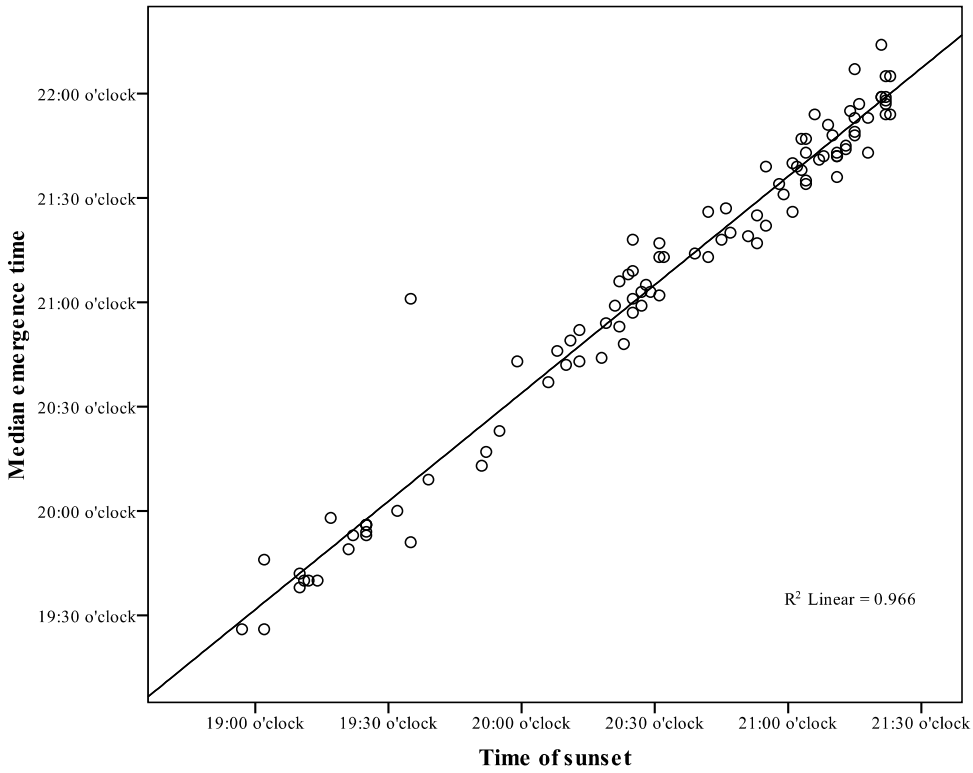

Figure 3.3: Correlation between the time of sunset and median bat emergence time.

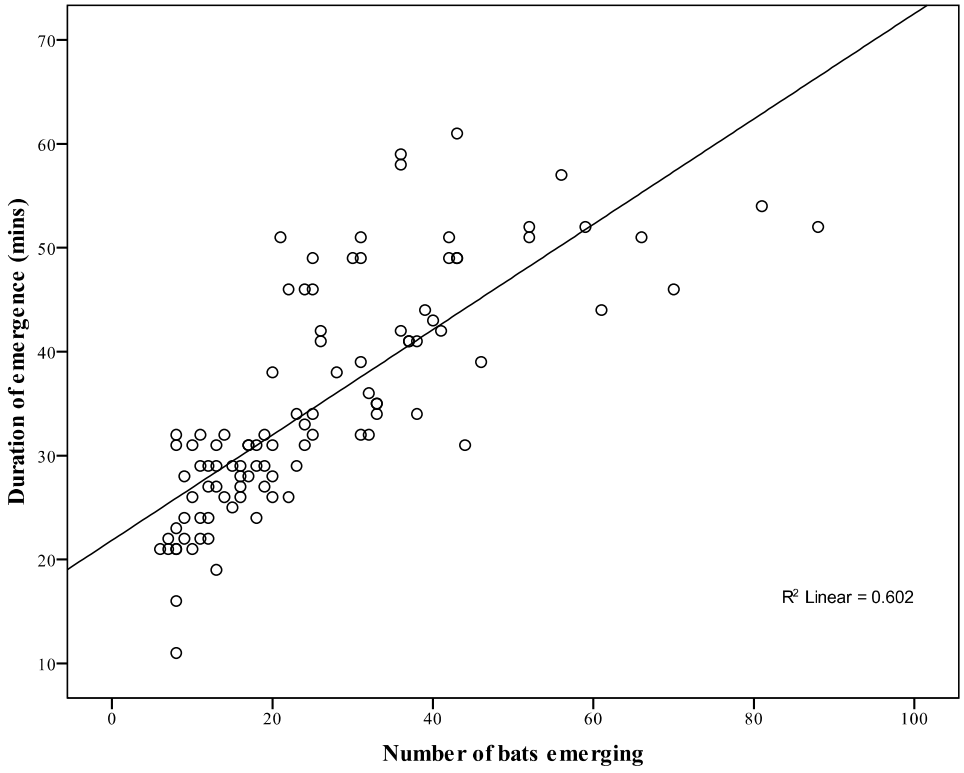

Figure 3.4: Correlation between the duration of the emergence and the number of bats emerging.

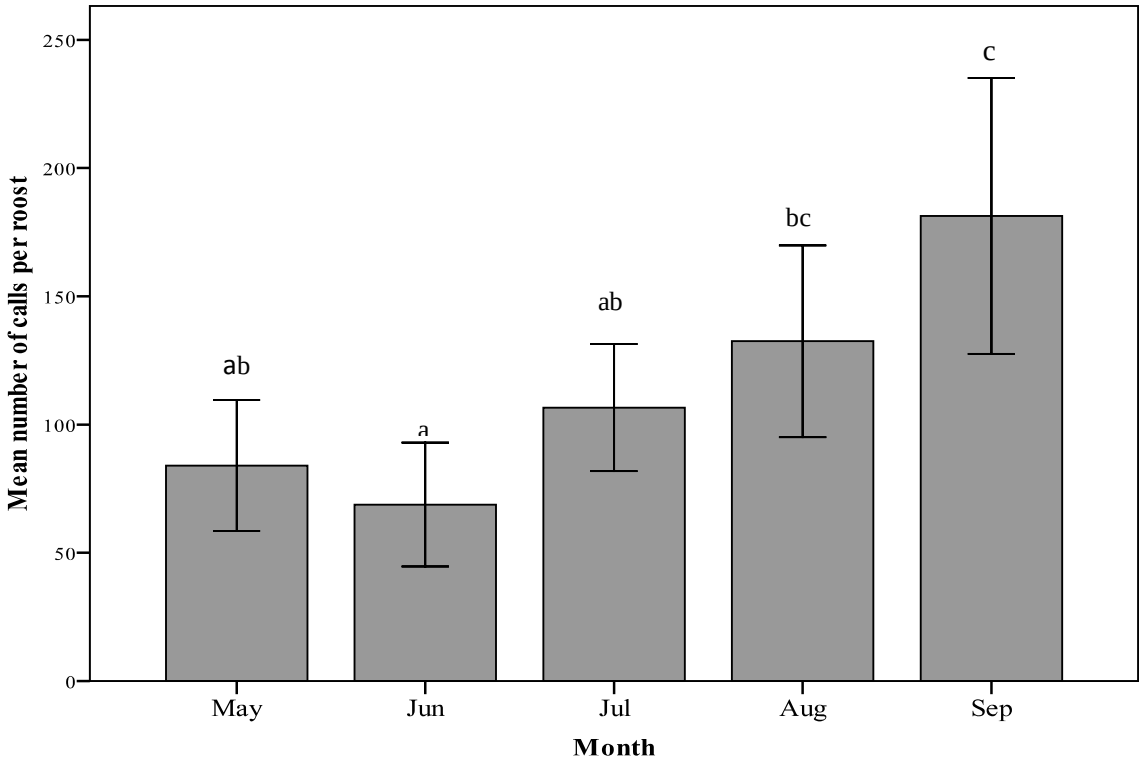

Figure 3.5: Mean monthly number of social calls recorded at twenty maternity roost sites in Sussex from May to September 2007. The error bars show the standard error of the mean. There was a significant difference in the monthly number of calls. Where the letters are different it indicates a significant difference between group means.

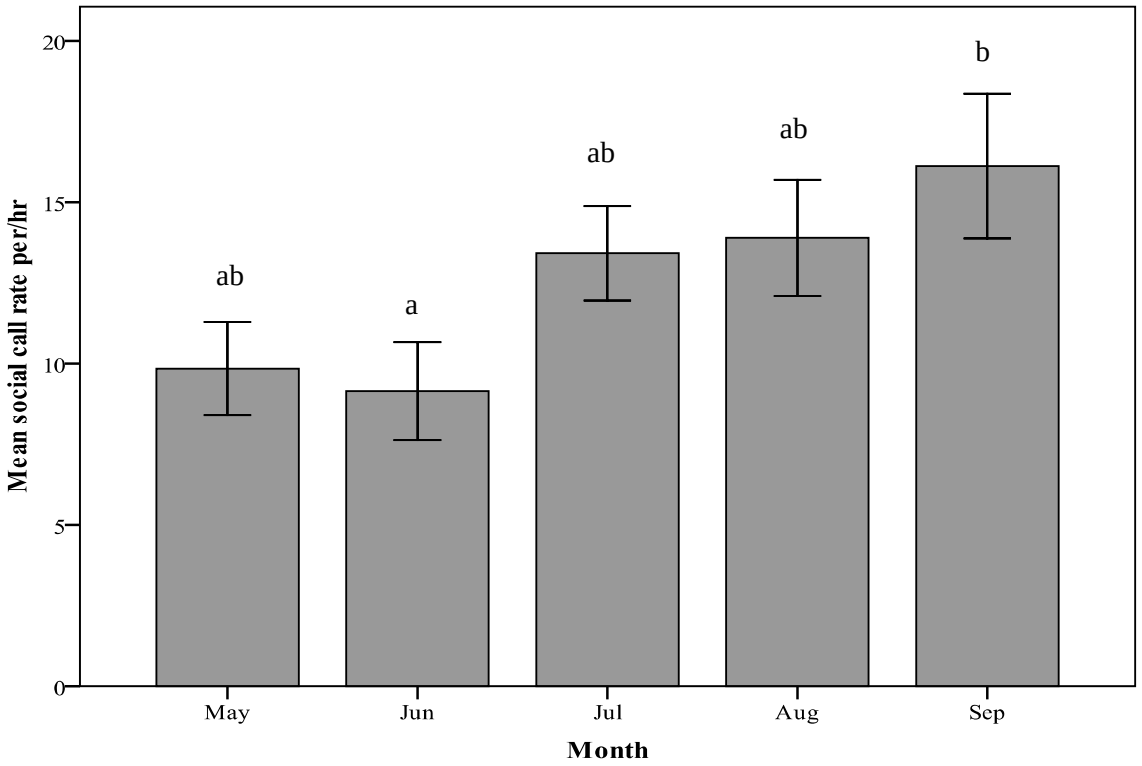

Figure 3.6: Mean number of social calls per hour recorded at twenty maternity roost sites in Sussex from May to September 2007. The error bars show the standard error of the mean. There was a significant difference in the monthly mean social call rate. Where the letters are different it indicates a significant difference between group means.

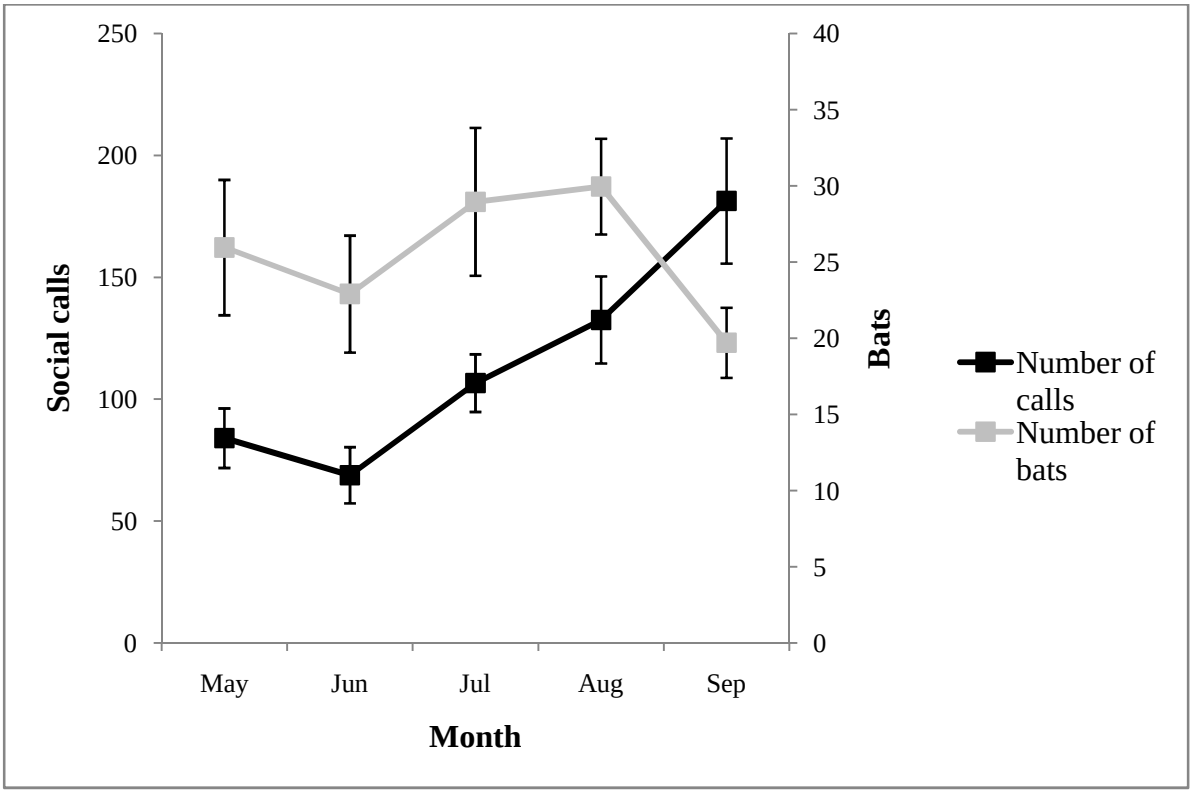

Figure 3.7: Relationship between the mean number of bats emerging and the mean number of social calls in each of five months. The error bats show the standard error of the mean.

Source of variation	**SS**	**SS/MS total**	**d.f.**	***P*-value**
Month	52483.34	26.20	4	< 0.001
Number of bats emerging	425180.58	212.27	43	< 0.001
Month*Number of bats	63768.46	31.83	33	0.525

Table 3.1: Results of the Scheirer-Ray-Hare test on the number of social calls recorded at maternity roost sites in relation to month and the number of bats emerging.

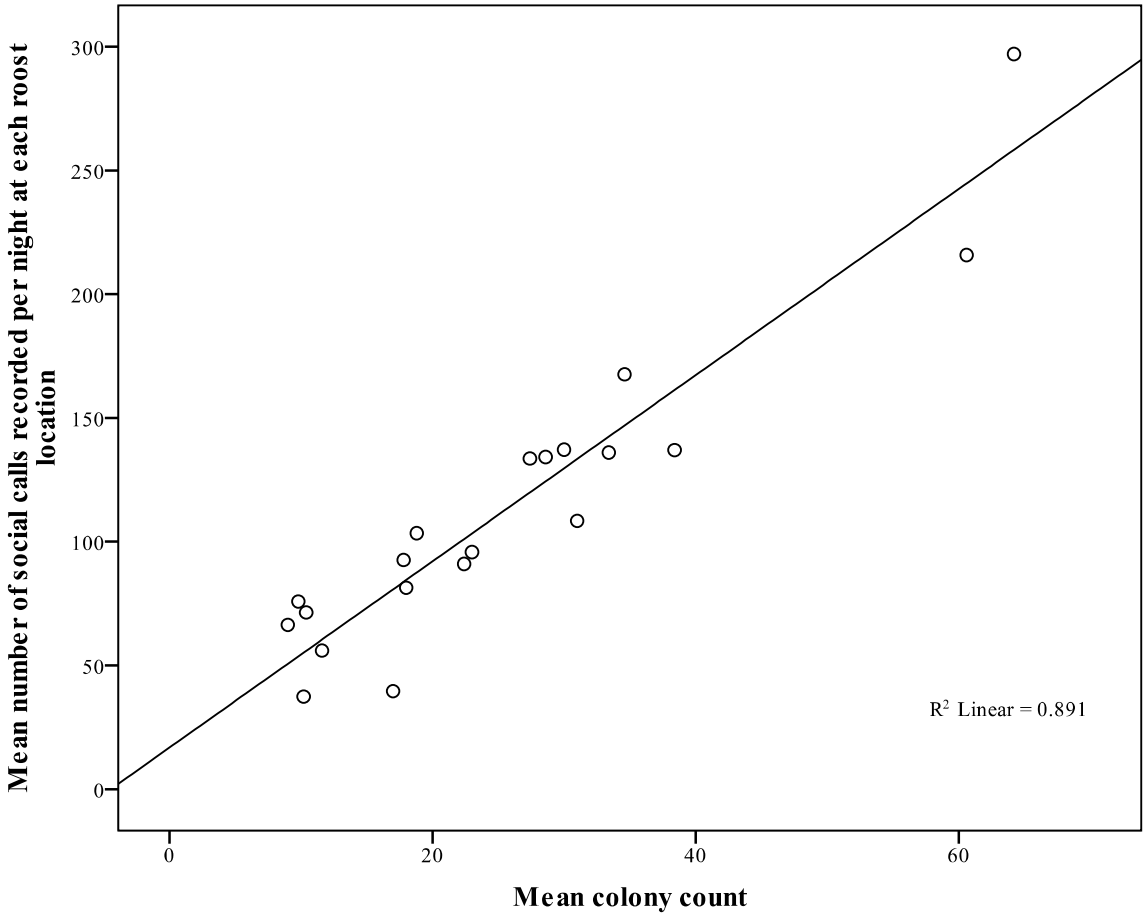

Figure 3.8: Correlation between the mean number of social calls recorded and the mean colony count.

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Source of variation	SS	SS/MS total	d.f.	*P*-value
Month	1994	0.315	4	0.988
Quarter	57176	8.99	3	0.029
Month*Quarter interation	2242	63.28	24	1.000

Table 3.2: Results of the Scheirer-Ray-Hare test on the number of social calls recorded at maternity roost sites in relation to the quarterly night period and the month in which the call was recorded.

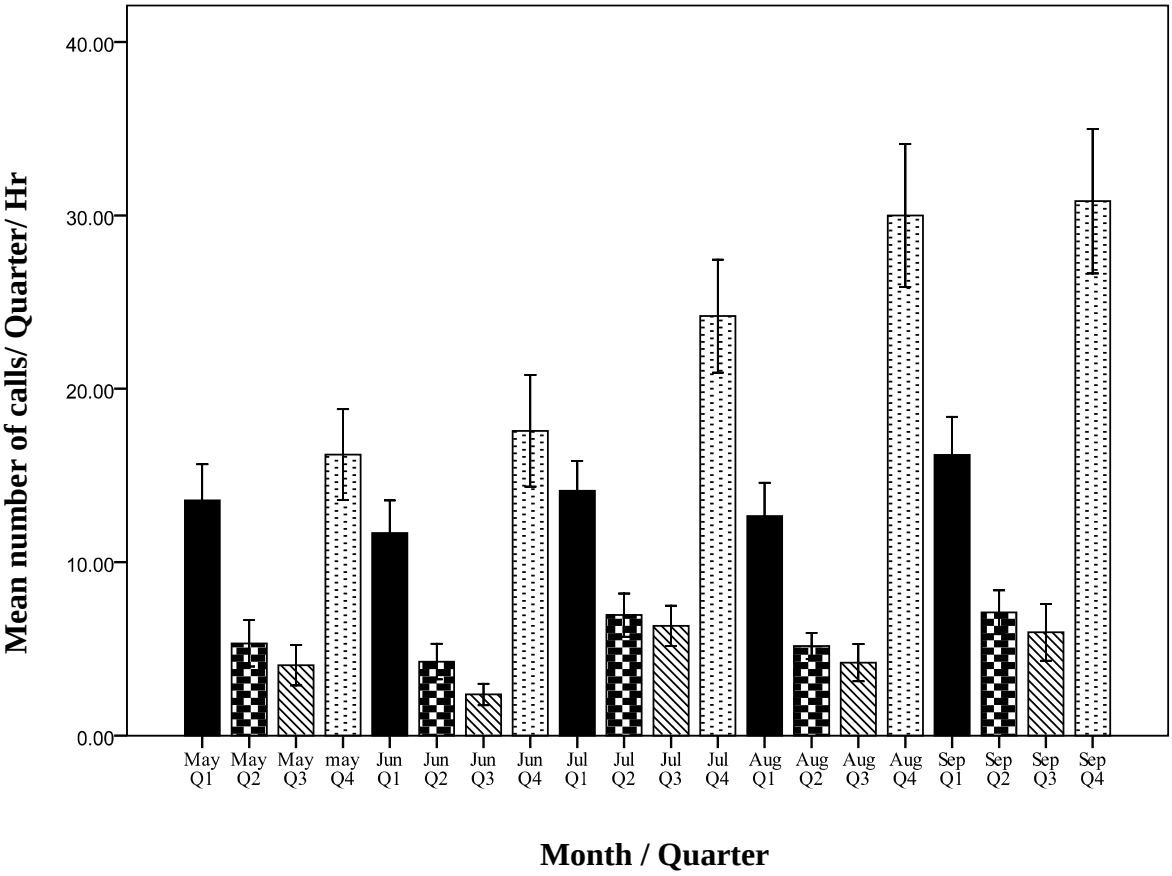


Figure 3.9: Mean number of *P. auritus* social calls per hour, per quarterly night time recording period for each month. The error bars show the standard error of the mean.

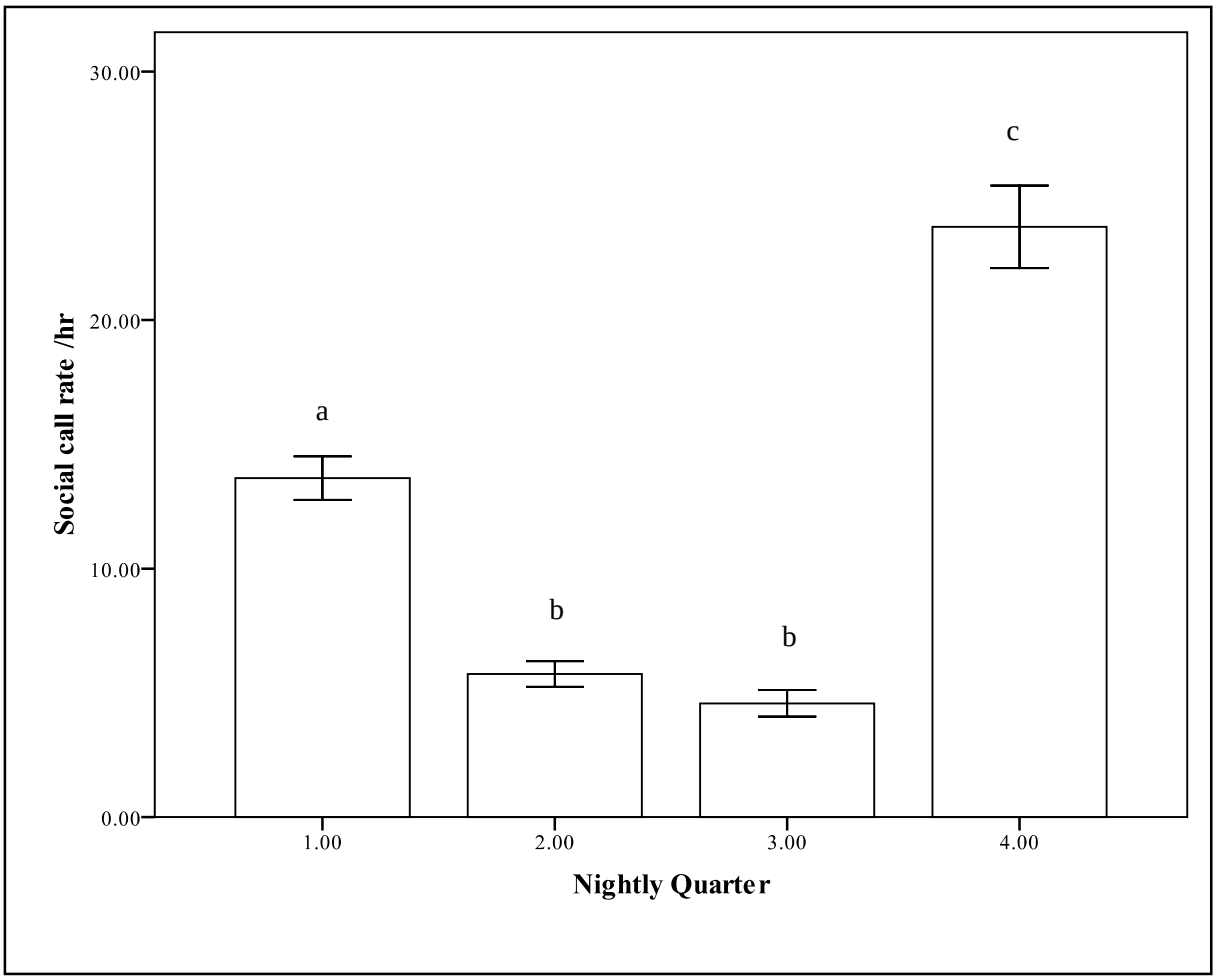


Figure 3.10: Temporal variation in the number of social calls recorded. Where the letters are different it indicates significant differences between groups.

CALLTYPE * MONTH Crosstabulation

			MONTH					
			MAY	JUN	JUL	AUG	SEP	Total
CALLTYP	1	Count	349	399	521	578	743	2590
		Expected Count	389.9	318.2	484.4	591.7	805.9	2590.0
		Std. Residual	-2.1	**4.5**	1.7	-.6	-2.2	
	2	Count	182	227	454	499	1038	2400
		Expected Count	361.3	294.9	448.8	548.3	746.7	2400.0
		Std. Residual	**-9.4**	**-4.0**	.2	-2.1	**10.7**	
	3	Count	260	69	112	156	181	778
		Expected Count	117.1	95.6	145.5	177.7	242.1	778.0
		Std. Residual	**13.2**	-2.7	-2.8	-1.6	**-3.9**	
	4	Count	353	136	287	343	406	1525
		Expected Count	229.6	187.4	285.2	348.4	474.5	1525.0
		Std. Residual	**8.1**	**-3.8**	.1	-.3	-3.1	
	5	Count	170	214	196	272	390	1242
		Expected Count	187.0	152.6	232.3	283.7	386.4	1242.0
		Std. Residual	-1.2	**5.0**	-2.4	-.7	.2	
	6	Count	357	319	506	688	696	2566
		Expected Count	386.3	315.3	479.9	586.2	798.4	2566.0
		Std. Residual	-1.5	.2	1.2	**4.2**	**-3.6**	
Total		Count	1671	1364	2076	2536	3454	11101
		Expected Count	1671.0	1364.0	2076.0	2536.0	3454.0	11101.0

Table 3.3: Results of Chi Sq test of association. Standardised residuals that have a positive value mean that the cell was over-represented in the actual sample, compared to the expected frequency. Using a critical value of Z =+/- 3.15, there are five positive (shown in blue font) and five negative (shown in red font) associations between call type and the month that the call was produced.

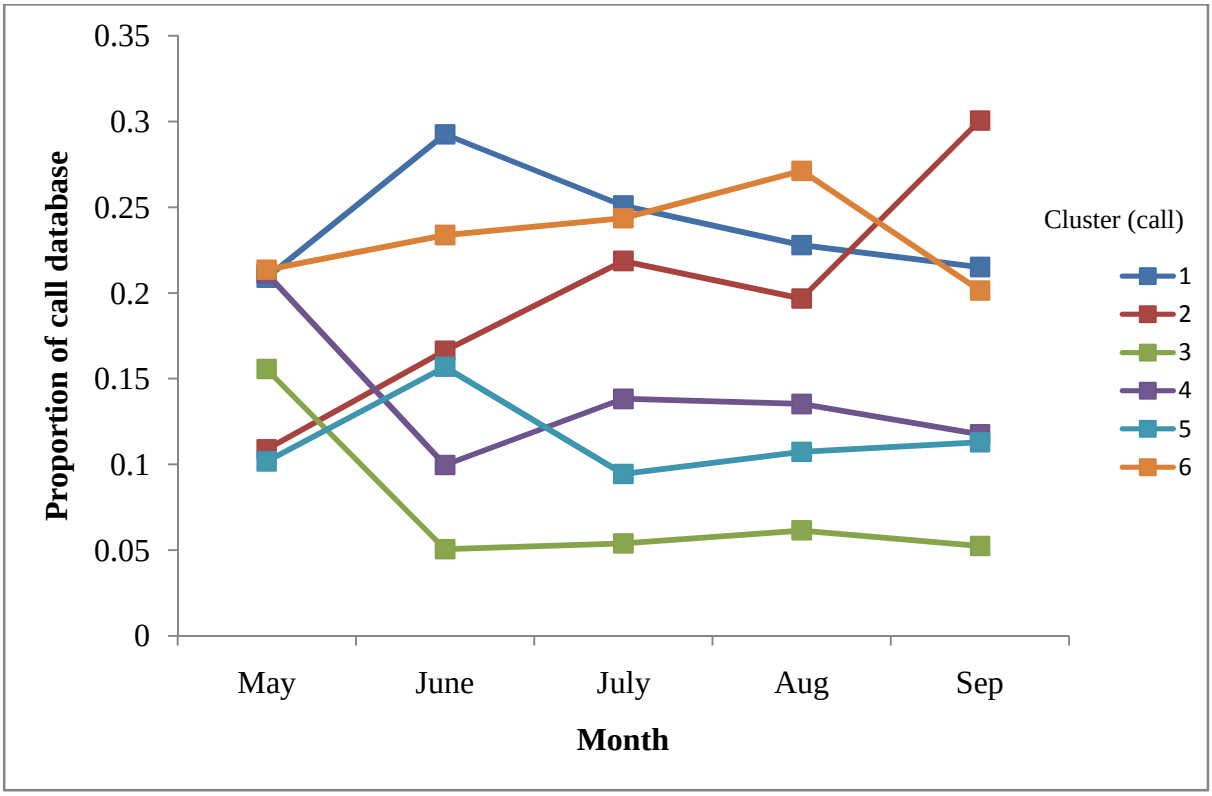


Figure 3.11: The proportion of each of the six call types (clusters) for 'Type A' calls recorded at maternity roost sites from May to September 2007.

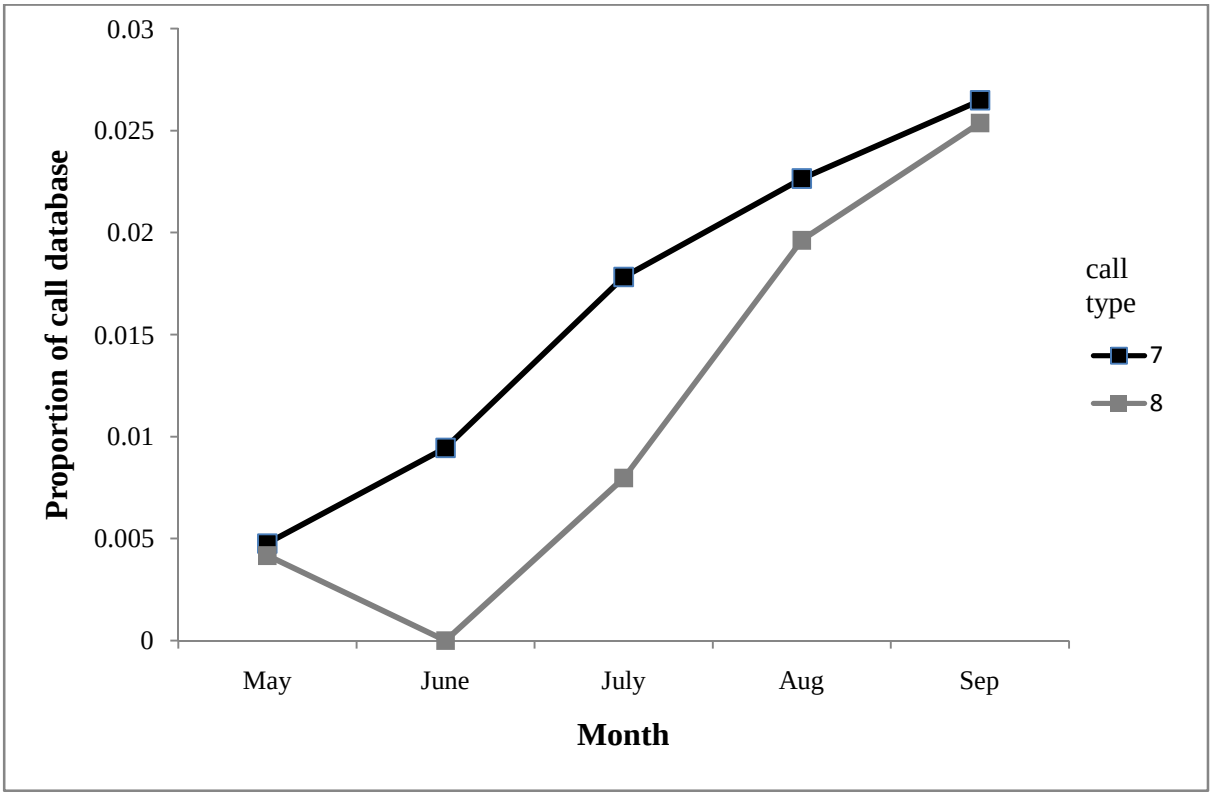


Figure 3.12: The proportion of call type 7 and 8 recorded at maternity roost sites from May to September 2007.

CALLTYPE * QUARTER Crosstabulation

			QUARTER				
			1	2	3	4	Total
CALLTYPE	1	Count	751	289	247	1303	2590
		Expected Count	682.4	326.4	257.3	1323.8	2590.0
		Std. Residual	2.6	-2.1	-.6	-.6	
	2	Count	530	341	227	1302	2400
		Expected Count	632.4	302.5	238.5	1226.7	2400.0
		Std. Residual	-3.0	2.2	-.7	2.1	
	3	Count	268	80	62	368	778
		Expected Count	205.0	98.0	77.3	397.7	778.0
		Std. Residual	2.4	-1.8	-1.7	-1.5	
	4	Count	418	231	179	697	1525
		Expected Count	401.8	192.2	151.5	779.5	1525.0
		Std. Residual	.8	2.8	2.2	-3.0	
	5	Count	279	158	131	674	1242
		Expected Count	327.3	156.5	123.4	634.8	1242.0
		Std. Residual	-2.7	.1	.7	1.6	
	6	Count	679	300	257	1330	2566
		Expected Count	676.1	323.4	255.0	1311.5	2566.0
		Std. Residual	.1	-1.3	.1	.5	
Total		Count	2925	1399	1103	5674	11101
		Expected Count	2925.0	1399.0	1103.0	5674.0	11101.0

Table 3.4: Results of Chi Sq test of association. Standardised residuals that have a positive value mean that the cell was over-represented in the actual sample, compared to the expected frequency. There were no positive or negative associations that reached the critical value of Z = +/- 3.09.

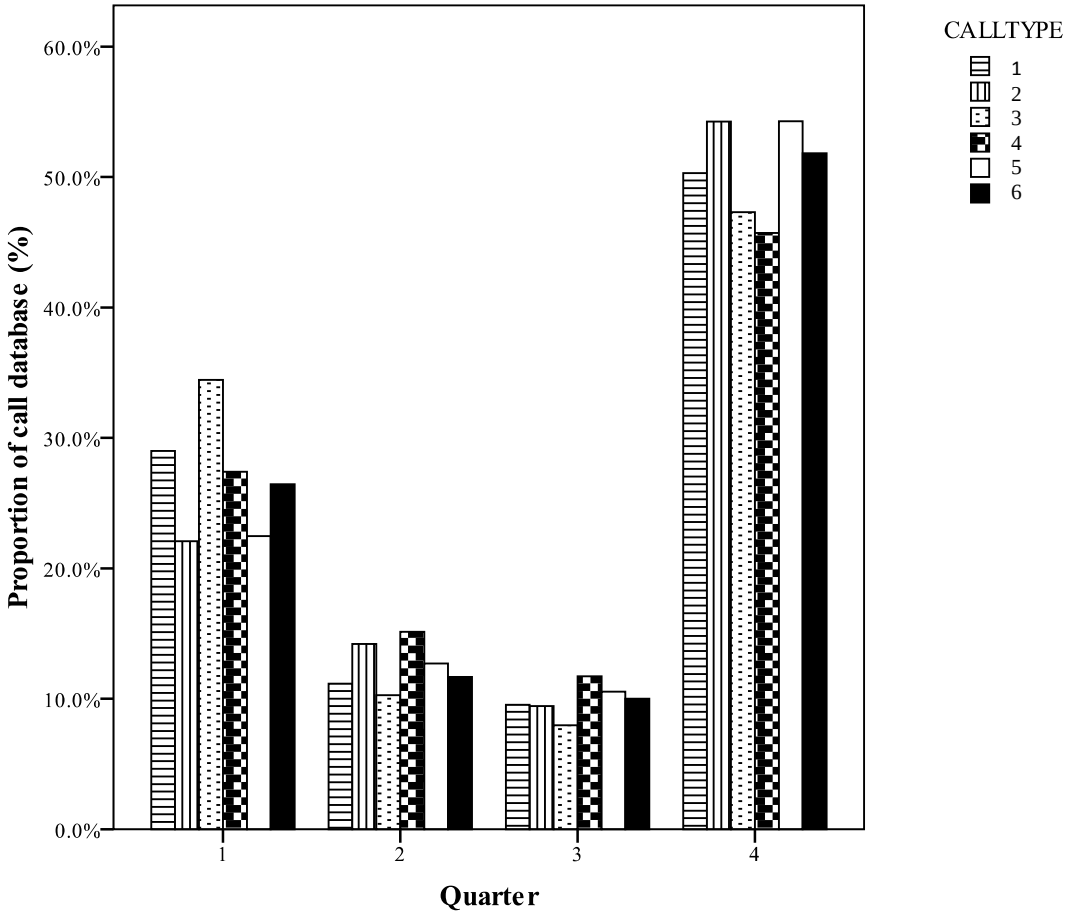


Figure 3.13: The proportion of all Type A calls that each of the six call types accounted for in each quarter.

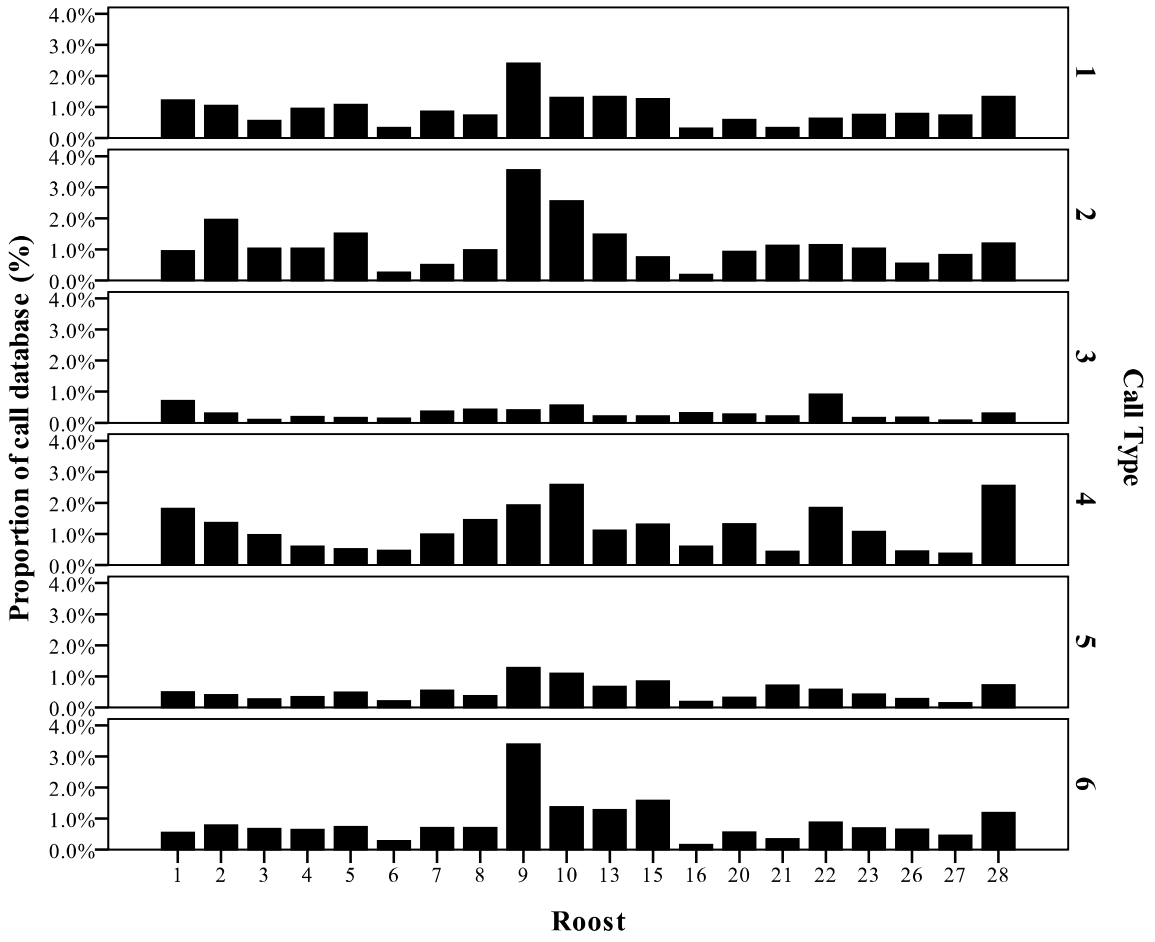


Figure 3.14: The proportion of Type A calls that each of the six call types accounted for at each of the 20 maternity roost sites across Sussex.

4.1 - Introduction

P. auritus produce a variety of social calls. These are produced frequently at maternity roost sites, particularly around the times that coincide with bats leaving or returning to the roost (as demonstrated in Chapter 3). Social calls are also given by bats away from the roosts but they are given much less frequently, but the functions of these calls are largely unknown.

One method of studying the function of vocal signals is to use playback experiments in which recordings of calls are broadcast, and responses of one or more animals to those calls are monitored. Playback techniques have been used to study call function in various animals, in particular birds (for review see Douglas and Mennill 2010) but, also in primates (Norcross et al., 1994; Semple, 1998; Smith et al., 2009), and other mammals, for example lions (McComb et al., 1993) and North American red squirrels *Tamiasciurus hudsonicus* (Digweed and Rendall, 2009). Playback has also been used to investigate the behavioural responses of bats to a variety of calls, including both echolocation (Barclay, 1982; Gillam, 2007; Jonker et al., 2010; Voigt-Heucke et al., 2010) and social calls (Balcombe and McCracken, 1992; Barlow and Jones, 1997; Russ et al., 1998; Wilkinson and Boughman, 1998; Knornschild and Von Helversen, 2008).

Conducting playback experiments at colony roost sites might be problematic because of the social complexity of the situation. There would be high levels of background noise from the bats' own calls, and it is likely that multiple bats would respond to the same stimulus, both of which might confound interpretation of the results. There is also the risk that playback

experiments would disturb the bats and lead them to abandon the roost. Playback experiments at foraging sites are less likely to face these problems.

Recording how *P. auritus* respond to playback of social calls at woodland sites may help us to understand the function of these calls away from roost sites. However, as it is often difficult to obtain high quality recordings of *P. auritus* away from roost sites (as social calls are produced so rarely), simulations of *P. auritus* social calls were used as an alternative method to produce high quality calls with little interference. The Autobat is a portable ultrasound synthesiser that can be used to simulate vocalisations of bats. It was originally developed to be used as an acoustic lure to attract bats for survey purposes, particularly in woodland habitats where bats are otherwise difficult to catch. Its effectiveness has been demonstrated experimentally (Hill & Greenaway, 2005), and it can be used to attract various species (Hill & Greenaway, 2009).

The experiments described in this chapter aim to investigate why bats respond to the Autobat stimulus by (i) comparing the bats' responses to simulated calls of their own species, another species and mechanical noises in a comparable bandwidth, (ii) assessing whether responses vary seasonally and between sexes, and (iii) analysing the nature of behavioural responses the bats show to a range of stimuli modelled on various social calls of their own species.

4.2 - Pilot Study: Filming behavioural responses to simulated social calls in the field.

4.2.1 - Introduction

The main aim of the pilot study was to determine the feasibility of recording behavioural responses of *P. auritus* to simulated social calls in the field, and whether it would be possible to obtain a usable estimate of how close a responding bat approached the source of the stimulus (Autobat speakers). The pilot study also aimed to identify a suitable latency interval for deciding when one response ended and another began.

4.2.2 - Methods

4.2.2.1 - Pilot study filming and data collection procedure

For the pilot study 18 recording locations were chosen at five sites, across two counties, in East and West Sussex. Recording locations at the same site were situated at least 200 metres apart in order to minimise the likelihood of the same bat being recorded at different locations. The Autobat speakers were suspended from a horizontal tree branch, at a height of approximately three metres, using metal hooks. The output from the Autobat was broadcast as ultrasound using two Senscomp/ Polaroid Series 600 Environmental Grade transducers. The output from these transducers is highly directional, with a beam of about 15^{O} at 50kHz, -6dB (Senscomp, 2004). One way of increasing the area over which the output is broadcast is to mount transducers on a pole and rotate the pole by hand (Hill & Greenaway, 2005), but it is not practical to do this for long periods, or when the behavioural responses of the bat are to

be recorded. To overcome this problem an automated system was built by Frank Greenaway that incorporates a rotating deflector to distribute the ultrasonic signal, as described below.

The circuitry is housed in a plastic box (12.5x8x3.5 cm). The transducers are mounted vertically, above the box, 8 cm apart and facing one another. Midway between them is a square vane (4.5x4.5cm) made of 0.5mm polished aluminium, which is attached to the spindle of a 12v electric motor. Power supply to the motor is controlled by a pulse-pause timer (Velleman Mk111) that allows both the pulse length and the interval between pulses of power to the motor to be adjusted. When the vane is perpendicular to the transducers it had no effect on the direction of the ultrasonic output. When it is parallel it reflects sound directly back to them. However, in all other parts of its rotation the vane serves to deflect the ultrasound of each transducer in a direction determined by the resting position. The resting position of the vane changes after each spin and is not controlled in anyway.

For the current study the interval between the start of each pulse was set at 2.5 seconds, and the pulse length was adjusted so that the vane rotated rapidly for about 0.25 seconds, during which time it completed three to five rotations before coming to rest. A test trial of 240 successive spins found that the number of times the vane came to rest in each of six 30^{o} sectors did not differ from what would be expected by chance (χ^2=7.15, df=5, p=0.210). Therefore, the system should produce a fairly even distribution of the ultrasound output in the area surrounding the speaker box.

Two Sony Handycam (Model DCR – DVD91E) digital video camera recorders were set up to record responses to the Autobat. One camera was placed directly beneath the speakers, on a tripod approximately 0.5 metres from the ground recording the bat's location in the horizontal plane, and the second camera was positioned approximately two metres away from the speakers, on a tripod approximately 1.5 metres from the ground, recording the bat's location in the vertical plane. Two infra-red lights (Model Envin micro 75L) were used to illuminate the area around the speakers. A synthesised social call, stimulus A, (Figure 4.1) comprised of the same sweep frequencies repeated five times, (but the third and fourth sweeps are attenuated) was then produced using the Autobat and the bats that responded to the stimulus were captured on video. Their vocal responses were recorded using a Petterson D240x bat detector and a Sony portable MD recorder (Model MZ-RH710). Using headphones with the D240x allows heterodyne output to be monitored in one ear, while the time expansion output can be heard in the other. Whenever a suspected social call was heard in heterodyne, the time expansion trigger was pressed, and 1.75 seconds of sound was played with a time expansion factor of ten. If the output included one or more social calls of *P. auritus*, it was then recorded onto the minidisc player. The Autobat broadcasted call A for a period of five minutes followed by an interval of five minutes of silence. A total of 33 two hour filming sessions were (each split between two locations at each site) took place from May 2006 to September 2006. Filming had to be curtailed on three occasions to a one hour session due to rainfall.

4.2.2.2 - Pilot study data analysis

The data from each filming session was downloaded to a personal computer (Dell Inspiron 2200) and analysed using Sony Picture Package Version 1.8 for DVD Handycam. An

inherent difficulty when recording and measuring behaviour is how to determine when one behaviour responses ends and another begins. The response, in this instance, refers to a bat being recorded on video in the experimental area regardless of whether a stimulus was being broadcast. For example, if a bat responds to a stimulus for a ten second period but flies away from the stimulus twice during that period, should that be recorded as a single ten second behavioural response or as three shorter behavioural responses? In principle, the shorter the interval the more accurate a time-sample recording will be. However, the shorter the interval the less likely it is to record reliably several categories of behaviour if they occur in unison, especially if the activity is complicated or occurs rapidly (Martin and Bateson, 2007). The frequency the responses were plotted against the interval. If behaviours are clumped, with many behaviours occurring within short intervals and few occurring at longer intervals, then the point at which the slope of the line changes most rapidly corresponds to the break point (Slater and Lester, 1982). Slater & Lester (1982) recommended that a slightly longer interval may be more accurate than the one corresponding exactly to the break point.

4.2.2.3 - Pilot Study Behavioural analysis

Once the response interval time was ascertained, each response was edited into a separate file and the behaviour observed and noted. This included the type of flight, a general impression of the size and shape of the responding bat. Ultrasound recordings were analysed using Batsound (v.3.3.1 Pettersson Elektronik AB, Uppsala) and the recordings were time matched with each response (where possible). A preliminary categorisation of behaviours was made, the results of which are not reported separately but which were used to inform the full categorisation of behaviour (detailed in Section 4.4.4.3).

4.2.2.4 – Estimating the proximity of the bat to the speakers

In order to test the feasibility of estimating the proximity of a responding bat to the speakers of the Autobat stimulus, using the speaker box as a gauge, a trial was conducted using a solid object (a suspended tennis ball) placed from 5cm to 100cm at intervals of 5cm from the centre of the speaker box (dimensions: 12.5cm width, 11cm length and 4cm depth) vertically, and then this was repeated horizontally. The resulting 40 positions of the ball were filmed in daylight and edited into separate files using Sony Picture Package Version 1.8 for DVD Handycam with a note made of the actual distances for each file. The 40 files were randomised and the distance of the ball from the speaker box was estimated for each of the 40 files. The estimated and actual distances for each file were subsequently compared in order to assess the error rate using a test-retest reliability statistic.

4.2.3 - Results

4.2.3.1 - Pilot Study: filming behavioural responses

A total of 33 video recording sessions were carried out across 18 sites from May 2006 to September 2006, which resulted in 66 filming hours. Bats frequently flew out of view for a brief period and then back into view again, so it was necessary to set a criterion by which to decide whether such occurrences would be counted as one response or two. The time interval was measured between each pair of successive records to the nearest second. Figure 4.2 shows the frequency distribution of interval lengths up to a maximum of 20 seconds, which

included 95% of all intervals between responses recorded in 2006. The break point, indicated by the red line, is approximately seven seconds but, according to Slater and Lester (1982, the best estimate is a slightly longer interval than the one corresponding to the break point, so a conservative estimate of nine seconds, indicated by the blue line, was applied as the criterion for minimum time interval between responses. This resulted in a categorisation of 208 separate responses that were used for analysis. There is the possibility that two short responses may have been categorised as one longer response. For example, a short response where a bat appeared on film for three seconds, an interval of five seconds with no bat on film, followed by a subsequent short response of three seconds where the bat was on film. This is unlikely to have occurred very frequently, as 83% of the times a bat flew into the view of the camera, the time intervals between successive responses were less than five seconds. Field notes also indicate that the responding individual more often than not flew in the vicinity of the Autobat during the response, but flew around trees, which may have resulted in the responding individual being obscured from the view of the camera lenses. Responding individuals may also have been missed from individuals approaching from behind the horizontal camera.

4.2.3.2 - Approximating the distance the bat approached the stimulus

The intra-observer reliability test coefficient indicated that the estimated distance of an object (the tennis ball) from the speakers, using the dimensions of the speaker box as a reference guide, gave a fairly reliable estimate of distance (Pearson Correlation $r = 0.992$, $n = 40$, $p < 0.01$). The estimates of distances were correct to 10cm all cases and, therefore, it was concluded that it was possible to estimate an approximate distance between the responding bat and the stimulus. Figure 4.3 shows a still photo of a bat responding to the stimulus within

12.5 cm of the speaker box in the vertical plane. Figure 4.4 shows a still photo of the same bat responding to the stimulus within 25 cm of the speaker box in the horizontal plane. Taken together, it was possible to estimate the approximate distance that a bat approached the stimulus.

4.2.3.3 - Responses to a P. auritus *simulated social call*

There were significantly more bat responses filmed, per hour, when the Autobat was broadcasting social calls than when the Autobat was silent (Wilcoxon n = 66, z = - 4.878, p=0.0001), shown in Figure 4.5. There was also a significant difference between season in the mean response rate per hour when the Autobat was broadcasting a stimulus social call (Kruskal-Wallis ANOVA H = 14.882, n = 5, p < .005). Post hoc pair wise comparisons were made (using Mann Whitney U with a Bonferroni correction to reduce Type 1 error rate (.05/10) resulting in a critical value for significance of Z = 2.81, p = 0.005) to investigate where the differences in response rate per hour were significant and the results are detailed in Table 4.1. There were significantly more responses per hour in both July and August compared to September and significantly fewer responses per hour observed in May compared to June, July and August. In contrast, there was no significant seasonal difference in the response rate per hour when the Autobat was silent in the two minute intervals between the three minutes of stimulus broadcast (Kruskal-Wallis ANOVA H = 2.372, n = 5, p = 0.668.). Furthermore, responses recorded in this period may be as a result of latency in response to the stimulus (i.e. some of the bats filmed in these intervals may actually have been attracted by the Autobat, but were reluctant to approach too close while it was broadcasting). Of the 29 responses that occurred when the Autobat was silent, 15 occurred

within the first minute of silence, three in the second, four in both the third and fourth minute and three in the fifth minute. Although there were more responses that occurred in the first minute of silence after stimulus A was terminated, this difference was not significant (Kruskal-Wallis ANOVA H = 3.535, df = 4, p = 0.473).

P. auritus were the most frequently recorded responding species, as shown in Figure 4.6, with a total of 143 responses (response rate per hour 4.81, SD = 3.22, n = 143), recorded from May to September 2006. Ultrasound recordings, both echolocation and social, were obtained for 73 of the *P. auritus* responding to the stimulus. The remaining 70 responses were identified as *P. auritus* on the basis of morphology (both ear and wing shape) and flight pattern (slow and fluttering). There was the likelihood that *P. austriacus*, if responding, could have been misidentified as *P. auritus*. However, this considered unlikely as *P. austriacus* had been captured at only one of the woodlands, Ebernoe Common, and this was a capture of a single individual. Furthermore, radio-tracking of the bats from the colony that this individual came from showed that this species had a distinct preference for more open habitats, such as meadow and pasture (Frank Greenaway & Daniel Whitby, pers. comm.). *P. austriacus* preference for more open habitats has also been reported by radio-tracking studies on the continent (Fluckiger and Beck, 1995; Sevcik, 2003). It should be noted, however, that the use of both mixed and deciduous woodland has also been reported (Barataud, 1990).

A total of 42 responses (1.27 per hour, SD = 1.15, n = 42) could not be identified to species level. This was because no ultrasound recording was obtained in these cases. 15 responses (0.42 per hour, SD = .66, n = 15) were identified as those of *Myotis* species. It was not possible to identify most species of this genus due to the overlapping parameters of their

echolocation calls. It was possible, however, to identify Natterer's bat *Myotis nattereri* as this species has lower end frequencies (i.e. bellow 20kHz) in their echolocations calls than other species in this genus (Russ, 1999; Jones and Waters, 2000; Jones and Holderied, 2007) Eight of the fifteen responses identified as those of the *Myotis* genus were therefore confirmed to be *M. nattererii*. Seven responses (0.21 per hour, SD = .54, n = 7) were identified as soprano pipistrelle *Pipistrellus pygmaeus* and 1 response (.03 per hour, SD = .17, n = 1) was identified as common pipistrelle *Pipistrellus pipistrellus.*

4.2.3.4 - Vocal responses to the stimulus

During the recording of behavioural responses on video, 73 recordings were made of *P. auritus* vocalisation sequences. Of these 46 were exclusively echolocation sequences, 22 included both echolocation and social calls, and five recordings were exclusively social calls. Therefore, a total of 27 out of the 73 *P. auritus* responses recorded (37.0%) included a social call that was recorded during the filming period. Figure 4.7 shows a sonogram of a *P. auritus* responding vocally to the Autobat.

4.2.4 - Discussion

The pilot study demonstrated that it was feasible to record behavioural responses of *P. auritus* to simulated social calls in the field using infra-red video and that it was possible to ascertain the approximate minimum distance between the responding bat and the stimulus.

The pilot study also provided the basis for setting nine seconds as the minimum interval required to classify successive observations as separate responses.

In this pilot study there was evidence of other species being attracted to the stimulus, in particular species from the genus *Myotis* and, specifically, *Myotis nattereri*, which suggested that, in addition to intraspecific responses, there may also be responses that represent interspecific responses. *M. nattereri* is a species frequently associated with woodland habitats (Parsons and Jones, 2003; Smith and Racey, 2008) and, like *P. auritus*, gleans to capture prey items (Siemers, 2001; Swift and Racey, 2002). However, in contrast to *P. auritus*, *M. nattereri* rarely uses prey-generated sounds to detect prey and is able to find prey by echolocation very close to vegetation (Siemers and Swift, 2006).

Approximately 20% of the responses could not be identified to species level in most cases because there was no time expansion recording made at the time of the bat responding. Less than 56% of the filmed responses of *P. auritus* had an ultrasound recording accompanying the responses and almost 37% of these recordings included a *P. auritus* social call. The use of an alternative recording system, such as a continuous automated ultrasound recorder, may achieve improved identification of responding bats and maximise the chances of recording vocal responses to the stimulus.

4.3 – Investigating the capture rates of *Plecotus auritus* to the Autobat using different stimuli

4.3.1 - Introduction

The pilot study showed that *P. auritus* responded to simulated social calls but there was evidence that other species also responded. Studies by Hill & Greenaway (2005, 2008) found that *P. auritus* was the species most frequently captured when simulated *Motis bechsteinii* social calls were used as an acoustic lure. These observations raise the question of whether bats approach the stimulus because of its resemblance to a social call of their own species, or if the response represents investigation of a novel source of ultrasound in the environment. If the similarity to a *P. auritus* social call is important, then the response rates to these calls should be higher than to social calls of other species, or to other stimuli of similar frequency and intensity.

Like many other species, *P. auritus* frequently responds to simulated social calls by rapidly approaching the speakers, which is why the Autobat is effective as an acoustic lure (Hill and Greenaway, 2005; Hill and Greenaway, 2008; Schöner et al., 2010). This means that capture rates may be used as a crude measure of responsiveness to different stimuli. An experiment was, therefore, conducted in which capture rates to simulations of *P. auritus* social calls were compared with those using other stimuli.

4.3.2 - Methods

Trapping took place at ten sites in six woodlands in West Sussex and Surrey. Each trapping site was used only once. Where multiple trapping sessions took place in the same woodland they were separated from one another by at least 200 metres. At each trapping site two harp traps were set in the interior of broadleaved woodland at least 150 metres apart. Harp traps were used in preference to mist nets as the traps could be left unattended for brief periods, which enabled the experimenter to change the calls and check the traps. Furthermore, harp traps are considered to be especially effective in catching small bats that weigh less than 30g (Kunz and Kurta, 1988; Francis, 1989).

Two Autobats were programmed with three different simulated stimulus calls and one Autobat was placed adjacent to each harp trap. The ultrasonic transducers (Polaroid Series 600 Environmental Grade) which broadcast the simulated ultrasound calls, were mounted on a pole so that they were parallel to the bank of strings on the trap. The experiment began at least one hour, but less than two hours, after civil twilight. The three stimulus calls, shown in sonogram Figure 4.8, were played in an irregular sequence of five sweeps (each sweep between 11 and 12 milliseconds in duration) following the same temporal pattern for each call, with the same intersequence interval. Each call was played for 30 minutes in rotation and the bats captured in response to each call type were recorded. A Fisher exact probability test (2 x 3 contingency table) was used to compare the capture rates of *P. auritus* to each of the 3 call types, using the statistical software SPSS®.

4.3.3 - Results

Plecotus auritus was captured in the harp traps when responding to simulated social calls of their own species on eight nights out of ten, compared with only two nights when capture coincided with simulated *M. bechsteinii* social calls, and one night when a capture coincided with the non-bat ultrasonic noises, as shown in Figure 4.9. Simulated *P. auritus* social calls were significantly more effective at capturing *P. auritus* bats than either simulated *M. bechsteinii* calls or ultrasonic non-call noises (Fisher Exact Probability 2 x 3 p = 0.0009).

4.3.4 - Discussion

P. auritus responded on significantly more nights to the simulated *P. auritus* social call than to the simulated *M. bechsteinii* call or to the non-call stimulus. This supports the hypothesis that the response of *P. auritus* to the stimulus is a social one elicited by the similarity of the stimulus to a conspecific's social calls. A study by Russ, Jones and Racey (2005) which played back distress calls of soprano pipistrelles *Pipistrellus pygmaeus* also found that bats responded significantly more frequently (based on the number of echolocation recordings) to the base calls as opposed to control calls (random noise) or calls that had been experimentally modified to differ in frequency, interval and repetition rate (Russ et al., 2005).

If the responses to the stimulus did represent an investigation of novel sound, then no significant difference would be expected in the frequency of responses occurring to each of the three stimuli. If it was a generalised response in which the bat investigates something that sounds like a bat social call, then similar levels of response to both of the bat call stimuli

would be expected. The fact that simulations of the own species calls were most effective suggests that the similarity to a conspecific's call was a key factor.

In this study *P. auritus* was captured in response to the *M. bechsteinii* call on one night only. However, an extensive two year study to survey for *M. bechsteinii* across southern England (using simulations of *M. bechsteinii* social calls) found that *P. auritus* were captured frequently in response to this call type (in 62% of the woodlands surveyed in 2005 and in 74% of the woodlands surveyed in 2006), which suggests that response to simulations of other species calls does occur frequently. This experiment was limited as only three stimuli calls were tested and it may be that other stimuli that do not resemble *P. auritus* social calls could be equally as effective. Furthermore, only the individuals that were captured in the harp trap were recorded and, therefore, responses that did not involve a close rapid approach would not have been recorded.

4.4 – Assessing the sex differences in responses to the Autobat

4.4.1 - Introduction

In studies of vocal communication that use playback experiments, knowledge of the sex of the responding individual can help to elucidate call function (Semple, 1998; Smith et al., 2009; Douglas and Mennill, 2010). In animals that are diurnally active, sex can often be readily determined from visible physical differences. However, because bats are small, fast-flying and active at night, it is generally impossible to determine the sex of an individual without inspecting it in the hand. Therefore, an inherent difficulty with studying infrared video behavioural responses of bats to simulated social calls in the field is that, not only is it

difficult to accurately identify the species, it is impossible to determine the sex of the responding individual using infra red video alone.

The pilot study (Section 4.2) showed that *P. auritus* frequently responded to simulated sequences of a *P. auritus* social call, termed 'stimulus A'. However, the sex of the individuals responding was unknown. The two main aims of this study were to (i) compare the sex composition of bats captured by the Autobat throughout the season and to (ii) analyse whether there are behavioural differences between male and female responses to the stimulus by differential marking of captured bats.

4.4.2 – Experiment 1: Sex differences in capture rate when using the Autobat

4.4.2.1 – Experiment 1: Methods

A capture study was conducted at Plashett Wood, Clayhill Wood and Hemsley's Rough in East Sussex. The capturing of the bats was carried out over an entire season (May to September 2007) to provide data on whether there are seasonal differences in relative capture rates of males and females. Eleven capture locations (the positions of which are shown in in Appendix A.4.1) were chosen in the three woodlands. The 11 capture locations were situated in the areas where the most *P. auritus* responses had been recorded in 2006 (and in trial filming sessions in April and May 2007) from a total of 17 locations across the three woodlands. The bats were captured at the woodland sites over 23 nights between May and September 2007 using a combination of harp traps and mist nets, to which they were attracted using the Autobat stimulus A. Each site was trapped twice, but site nine was netted three

times as a high number of bats were captured at this location. Trapping was suspended for two weeks around the birth period, to avoid capture of bats in late pregnancy or early lactation.

4.4.2.2 – Experiment 1: Results

A total of 32 female and 20 male *P. auritus*, (detailed in Appendix A.4.2), were captured across the 11 different locations in the three woodlands in 2007. There were more female bats captured (a total of 32 over 23 nights of netting and trapping) than males (a total of 20 over 23 nights of netting and trapping) but this difference was not significant (Mann Whitney U test $Z = -1.772$, $n = 58$, $p = 0.076$). However, when the mean monthly capture rate per session for both males and females was plotted, as detailed in Figure 4.10, it was clear that there was a seasonal difference in the captures of males and females. A two-way ANOVA was carried out to test the effect of sex and month on the number of captures. This showed that there was no significant difference in the capture rates of bats for each month and no significant difference between the capture rates of males and females (as indicated above), but there was a significant effect of an interaction between month and sex, as shown in Table 4.2.

4.4.3 – Experiment 2: Differential marking of male and female bats

4.4.3.1 – Experiment 2: Methods

Each of the individuals captured (as detailed in Appendix A.4.2) was marked with a reflective metallic band. Marking each sex with a reflective band on opposite wings, allows for differentiation between males and females responding to the Autobat on infra-red film,

should they subsequently respond to the stimulus and be captured on film. In addition to the bats captured at the 11 woodland sites, seven female and four male bats were captured from a maternity roost in a house at Plashett Park Farm. This roost location was found during a pilot study in Plashett Wood in 2006 in which bats were captured and radio-tracked back to their roost (see Chapter 5). The bats were captured in a pole trap as they exited the roost. This roost was only trapped once, using this method, as frequent trapping at roost sites could potentially cause roost abandonment (Mitchell-Jones and Mcleish, 2004).

Aluminium-alloy 3mm bat rings (Mammal Society London) were covered in cyanoacrylate adhesive and a piece of reflective tape (Sakuma Retro Reflective Tape) was attached to each of the metal bands. One disadvantage of reflective tape to bands is that it is often chewed off by the bats, making regular replacement necessary (Kunz, 1996). Therefore, at least five coats of non-toxic water based varnish (Acquarella) was applied to seal the tape on to the band and reduce the likelihood of the bats removing it.

Female bats had a reflective wing band fitted around their right forearm and male bats had a reflective wing band fitted around their left forearm. After each night of capture at a site where bats were marked, a night of filming responses to the Autobat (over a period of two hours per night) followed within a minimum of five days after the capture took place. The filming of responses was carried out using the methodologies (detailed in Section 4.2.2.1) and the number of responses from bats with reflective banded wing bands was noted.

4.4.3.2 – Experiment 2: Results

A total of 44 hours of filming resulted in 39 responses of individuals with reflective bands which included 34 female responses, five male responses and 254 responses of *P. auritus*

with no reflective band. This resulted in a mean response of 0.866/hr of banded bats, which was lower than the 5.77 *P. auritus* responses per hour recorded for non-banded bats. Of the 34 responses by females, 25 were by seven radio-tagged animals. This was because these radio-tagged individuals were targeted by broadcasting the Autobat within their home range (see Chapters 5 and 6 for detailed analysis of radio-tracking study and how radio-tagged females responded to the stimulus). The remaining nine responses were recorded at five different sites (one response at Clayhill Wood, six responses at three sites in Plashett Wood and two responses at Hemsley's Rough). The male responses were recorded at two locations (four responses recorded at Clayhill Wood (all on the same night) and one response at Hemsley's Rough site 15). The low response rate recorded for males with reflective bands means that statistical comparison with responses of marked females was not possible.

4.4.4 - Discussion

There was no overall significant difference in the capture rate between the number of male and female bats. There was, also, no overall significant difference between months in the capture rates for both sexes. However, female bats were caught significantly more frequently earlier in the season and male bats were caught significantly more frequently later in the season. This seasonal difference between male and female bats has been observed in capture studies of bats in forests, without using lures. For example, an eight year study of mist netting study in the Ouachita Mountains in Arkansas found that the sex ratios of eastern red bats *Lasiurus borealis* captured were predominately male in late summer but were dominated by females in mid-summer (Perry et al., 2010). The authors suggested that the greater proportion of females found mid-summer may have reflected an increase in activity of females due to the demands of reproduction.

25 of the 34 responses by females (over 73%) were from seven bats that had a radio-tag fitted as well as a reflective band fitted. The remaining nine responses were recorded at five separate locations, which, based on radio-tracking data, are more than likely to have been responses recorded from at least five different females. This could mean that at least 12 of the 39 females (approximately 30%) were subsequently recorded responding to the stimuli on infra-red video after they had been captured and fitted with reflective bands. However, by comparison, a total of five male responses were recorded at only two sites (and four of the responses were recorded on one night, which could easily have been multiple responses of the same bat). This could mean that possibly only two of the 24 males (approximately 8%) were subsequently recorded responding to the stimuli on infra-red video after they had been captured and fitted with reflective bands, suggesting that, in comparison to females, males may be less likely to respond to the stimulus, perhaps as a result of been captured previously or, potentially because they may have larger foraging ranges.

Reflective bands, to enhance the visibility of flying bats at a distance or while in the roost, have been used with varying degrees of success by bat researchers. Some studies have found that the use of reflective bands was successful in identifying foraging bats at a distance (Bradbury and Vehrencamp, 1976; Humphrey et al., 1977; Racey and Swift, 1985; Rydell, 1989). However, these were for bats that tend to use more open habitats to forage than *P. auritus*. A study that compared the foraging habitats of *P. auritus* and *M. daubentonii* occupying the same roost, found that the bats marked with reflective bands could only be seen at short distances (Swift and Racey, 1983).

The use of metallic bands to mark bats needs careful consideration as there are potential welfare implications. Metallic bands can affect mobility and even cause direct injury if

inappropriately applied. However, Dietz et al., (2006) compared published and unpublished injury rates of 28 bats species and found that the injury rate for European vespertilionid bats, including *P. auritus*, marked with metal bands since the 1990's was generally below 5%. Only *Pipistrellus nathusii* showed a higher rate of injury at 6.3%.

The differences in behavioural responses of male and female bats may be better addressed using alternative methodologies (such as, for example, radio-tracking) given the low-recapture rate of bats with reflective bands responding to the Autobat on infra-red video and, also, the potential harm to the individuals (although this was considered to be low). It may have been possible to have captured a high proportion of the bats at known maternity roost sites surrounding Plashett Wood but, based on colony counts (see Chapter 3), this would have been in the region of 200 individuals and it was considered that this may have been too invasive in terms of the local population. It may be possible, however, to apply the technique (using the Autobat) in smaller woods, as there would be a greater probability of ringing a higher proportion of the population, yielding more informative results.

4.5: Behavioural responses of *P. auritus* to a variety of simulated social calls.

4.5.1 - Introduction

The pilot study (Section 4.2) used a single stimulus 'stimulus A' and study two (Section 4.3) showed that simulations of an individual call from the 'stimulus A' sequence was more effective at catching *P. auritus* than a simulation of an individual call from a *M. bechsteinii*

call sequence or a simulation of an ultrasonic noise. However, *P.auritus* used a wide variety of social calls, as demonstrated by the variety of call types recorded at the roost sites (as detailed in Chapters 2 and 3). Various calls have also been recorded in the field away from known roosts sites. Six of these calls had been found to be effective as stimuli for the Autobat acoustic lure (unpublished observations by Hill, Greenaway and Murphy). The main aim of this experiment was to assess whether differences were apparent in the nature of the bats' responses to the different types of synthesised call, whether these responses varied seasonally and whether changing the volume of the calls influenced the bats' responsiveness.

4.5.2 – Methods: Filming behavioural responses to a variety of stimuli

4.5.2.1 – Experiment set up and data collection procedure.

A total of 38 filming locations were selected in 11 woods in East and West Sussex and Surrey. Filming locations in the same wood were situated at least 200 metres apart in order to minimise the likelihood of filming the same bat at different locations. The experimental set up followed the procedure previously described for the pilot study (Section 4.2.2.1) with the following modifications: (i) the second camera, mounted horizontally, was placed three metres away from the stimulus in order to increase the filming area and capture more prolonged, complex behaviours; (ii) an automated broadband ultrasound recorder was used to continuously record all ultrasound in the vicinity of the Autobat speakers to improve the identification of species of bats responding; and, (iii) each call type was played for a period of three minutes with a two minute interval of silence between each call. This was to allow a sufficient latency period between calls (to correctly assign the response to the appropriate call), to ensure equipment was still recording and change film discs and batteries when

required and, also, to allow time for a sufficient variety of calls to be used at each filming session. A total of 172 hours of filming was carried out across the 38 locations from May 2007 to September 2007 and May 2008 to September 2008.

4.5.2.2 Simulated social calls

Six social calls were used as models for the stimulus simulations (calls A, C and D were recorded by David Hill & B, E, and F were recorded by Stephanie Murphy from free flying bats away from roost sites in 2006). For each stimulus the intervals between individual sweeps in a sequence were based on those of actual recordings, but the sequences themselves were repeated at much higher rates than have been recorded in the field. Ultimately, the patterns and rates of the sequence repetition were ones that had proved effective in attracting bats for capture. Consequently, it could be regarded as a super-stimulus, rather than an attempt to simulate the natural communication between *P. auritus*. Sonograms of the six stimuli used are shown in Figure 4.1 (stimulus A previously shown in Section 4.2) and Figures 4.11 to 4.15 (stimuli B – F). The calls differed in intensity and temporal patterning as well as structure. The intensity of each call was measured at the frequency of maximum energy for that call. The method by which intensity was measured and the equipment was calibrated is described in Appendix A.4.3. The acoustic parameters, temporal patterning and intensity measurements for each of the stimuli are detailed in Table 4.3.

Each stimulus was played for three minutes followed by an interval of two minutes of silence, and then another stimulus was played. All stimulus types were played both amplified and non-amplified (so that stimulus A with amplification was AL (loud), and stimulus A without

amplification was AQ (quiet)). The 12 stimuli were played in a randomised order (using the random number generator function in excel) changing the order that the stimuli were played each night. After a period of one hour, each stimuli having been played once amplified and once unamplified, the filming was terminated, the location moved, and the process repeated.

4.5.2.3 - Behavioural categorisation

The data from each filming session was downloaded to a Personal Computer (Dell Inspiron 2200), edited using Sony Picture Package Version 1.8 for DVD Handycam, and analysed in slow motion using VLC Media Player Version 1.1.4. For each night's filming the following details were recorded for each occurrence of a bat recorded on video: (i) the time of occurrence; (ii) the length of time for which the bat was visible; (iii) the estimated distance of closest approach to the speaker box; and (iv), a preliminary description of the behaviour. The edited behavioural sequences were collated and then analysed, repeatedly, with a view to placing each into a behavioural category.

The observed behaviours were separated into discrete categories, ensuring that the definitions of behaviour were clear, comprehensive and unambiguous. When choosing the observational categories that a response should be allocated to, the following guidelines (as recommended by Martin and Bateson 2007) were adhered to: (i) enough categories were available to describe each response in detail; (ii) each category was precisely defined and summarised with as much information as possible about the behaviour; (iii) categories were discrete (two or more categories did not describe the same thing); and (iv) behaviours within categories were uniform. After numerous examinations of the recorded behaviours and trials of

categorisation systems using 'within observer' reliability techniques to check each outcome (Martin and Bateson, 2007), a dichotomous key was constructed, as shown in 4.16. The description for each category was defined using criteria that could be readily understood by people unfamiliar with bats or their behaviour. Behaviours recorded during the experimental period are termed as a "response" below, regardless of whether the stimulus was playing or not.

4.5.2.4 - Assessment of the classification system

In order to assess the suitability of the behavioural categorisation system, an inter-observer reliability test was conducted using five scorers. Training scorers to allocate behavioural observations to specific categories is often difficult, yielding inconsistent results if the descriptions of categories or behaviours are open to interpretation by the scorer. The use of a binary key reduces the 'training' process but the scorers were given a brief training session, working through two filmed responses and using the binary key with the author. The scorers included three people with backgrounds in biological science (one post-doctoral and two PhD candidates) and two people with little or no scientific background. None of the scorers had any previous experience of studying bat behaviour.

The scorers were provided with a score sheet (shown in Appendix A.4.4), a definition sheet (shown in Appendix A.4.5), and the behavioural binary key (as shown in Figure 4.16). Each observer was given the same 50 edited behavioural observations, and these 50 observations were then split into two sets of 25 that were undertaken as separate tasks to reduce the risk of observer fatigue. The 50 observations chosen included eight from each of the categories (as

defined by the author in Figure 4.16) to ensure that there was a uniform distribution of the behavioural observations assigned to each category type (chosen from each category using a randomised number table) and two behavioural observations that could not be assigned to a category by the author. The scorers were asked to categorise them to category level (not subcategory level). The 50 observations were categorised by the author (using the binary key Figure 4.16) and the categorisations of the responses by the scorers were subsequently compared to the author's to ascertain the level of agreement using a binary scoring system, whereby 1 was agreement and 0 was non-agreement. Cochran's Q test was then used to test whether the scorers' classifications of the 50 behavioural responses were in agreement with the author's classifications.

4.5.2.5 - Data Analysis

All the data were entered into SPSS, a software package for statistical analysis. Non-parametric tests were used throughout because categorical data was included and the responses could not be assumed to be normally distributed.

4.5.3 – Results: Filming behavioural responses to a variety of stimuli

4.5.3.1 - Number of bat responses

A total of 87 video recording sessions were carried out across 38 sites during May to September 2007 and during May to September 2008, which resulted in 172 hours and 681 bat responses that were used for analysis. Figure 4.17 shows the mean response rate / hour, for

each month, for when the Autobat was broadcasting the six simulated *P. auritus* social calls and for when the Autobat was silent. There were significantly more bat responses recorded, per hour, when the Autobat was broadcasting social calls compared to the number of responses recorded when the Autobat was silent, and this was significant for each month of recording for both years (May to September) (Kruskal-Wallis ANOVA, $H = 6.818$, $df = 10$, $p = 0.009$.).

It was possible to identify the species or genus of the bat for 627 of the 681 responses recorded. *P. auritus* were the most frequently recorded species with a total of 381 responses recorded on film, followed by 105 responses of species from the genus *Myotis* (*M. bechsteinii, M. daubentonii, M. brandtii, M. mystacinus, M. alcathoe, M. nattereri*), and 86 confirmed responses of *M. nattereri.* There were also 30 *P. pygmaeus*, 19 *P. pipistrellus* and six *N. noctula*. The remaining 54 responses could not be identified to species level (the majority of these were bats seen at a distance on film but there was no corresponding ultrasound recording to assist in identification).

4.5.3.2 - Species response rates per hour

There was a significant difference in the response rate per hour between species when the Autobat was broadcasting the six simulated social calls (Kruskal-Wallis ANOVA $H = 245.640$, $n = 6$, $p < 0.001$), as shown in Figure 4.18. Post-hoc analysis (using Mann-Whitney U with a Bonferroni correction to reduce Type 1 error rate (.05/7) resulting in a critical value for significance of 0.007) was carried out to determine whether the differences were

significant and this revealed that *P. auritus* had a significantly greater response rate per hour compared to any other species, as shown in Table 4.4. Species from the genus *Myotis* and responses from bats that could not be identified to species level had significantly greater response rates per hour than *Pipistrellus pipistrellus*, *Pipistrellus pygmaeus* or *Nyctalus noctula.* In turn, *Pipistrellus pipistrellus* and *Pipistrellus pygmaeus* had significantly greater response rates per hour than *Nyctalus noctula* (test statistics detailed in Table 4.4). Occasionally echolocation calls of *Eptesicus serotinus* and/or *Nyctalus leisleri*, *Barbastella barbastellus* and *Pipistrellus nathusii* were recorded at some of the woodland sites. However, these calls did not coincide with any filmed responses of bats.

4.5.3.3 - Behavioural classification system

Six behavioural categories were defined, with a seventh category (Unclassifiable) added for responses that could not be assigned to any of the six. Each of the behavioural categories was further divided into subcategories according to the details of the behaviour, such as how close the bat approached the stimulus, or whether a behaviour was performed just once or repeated. This produced a total of 23 subcategories. The Cochran's Q test indicated a good level of agreement (96.2%) between the scorers and the author in categorisation of behavioural responses to category level (Cohran's $Q = 113.392$, d.f. 49, $p < 0.0001$), which indicated that the observed filmed behavioural responses could be consistently categorised.

4.5.3.4 - Behavioural responses of all bats responding to the stimuli

Of the 681 responses recorded on video, 654 could be classified into one of the main behavioural categories one to six. The remaining 27 responses (less than 4%) could not be satisfactorily assigned to one of the six main categories and were consequently assigned to category seven 'unclassified'. The responses that could not be categorised were 'unclassifiable' for a number of reasons including: difficulty in judging how near to the stimulus the bat approached; occasions when two or more bats responded together, thereby confounding the results; or occasions when the observed behaviour could not be assigned to a category with complete certainty.

4.5.3.5 - Analysis of P. auritus *behavioural responses*

The responses of *P. auritus* bats included all seven behavioural categories, as shown in Figure 4.19, whereas the behaviour of other species that responded to the stimulus could be categorised into only four categories. Notably, the three behavioural categories that included hovering were unique to *P. auritus*. A chi square test found that there was a significant association between species and response type ($\chi^2 = 185.694$, d.f. 19, $p < 0.001$), but when *P. auritus* was excluded from the analysis no significant difference was found ($\chi^2 = 14.056$, d.f. 19, $p = 0.652$). The 34 responses where it was known that the responding individuals were female (see Section 4.4.3.2) were compared to the remaining 254 responses to examine if there was a significant difference between the two groups of responses. There was no significant difference between the frequencies of behavioural categories for known females and responses for where the sex was unknown (Kruskal-Wallis ANOVA H = 0.115, d.f. = 1,

p = 0.734). A total of 87 (23%) of the filmed responses were of multiple (two or more) *P. auritus* responding simultaneously to the stimulus.

4.5.3.6 - Seasonal variation in P. auritus *response type*

There was evidence of differences in the mean number of responses between behaviour categories, evidence of seasonal differences and, also, evidence of an interaction between season and behavioural response type, as indicated by the results of the Scheirer-Ray-Hare test, shown in Table 4.5. A total of 595 post-hoc pair-wise comparisons were undertaken (using Mann-Whitney U with a Bonferroni correction to reduce Type 1 error rate (.05/35) resulting in a critical value for significance of p = 0.0014) to investigate where the differences were significant. Figure 4.20 details the results of the mean number of responses for each behaviour category in each month.

Excluding 'unclassified' behaviours, there was evidence of seasonal differences in the mean number of responses for each category. In May, category one responses 'approach the stimulus and leave immediately' were observed significantly more frequently than category six responses 'hover and leave'. In June, category four and five responses 'fly near the stimulus moving continuously' and 'fly near stimulus pausing to hover'(behaviours longer in duration), were observed significantly more frequently than responses assigned to categories one and six 'approach stimulus, leave immediately' and 'hover, leave immediately' (behaviours which are shorter in duration). In July, category two and five responses 'fly near stimulus completing at least one 360^{o} rotation' and 'fly near stimulus pausing to hover' were

observed significantly more frequently than responses assigned to categories four and six. There were no significant differences in the mean between categories for observed responses in either August and September.

4.5.3.7 - The effectiveness of stimuli of varying intensity

There were differences in the overall effectiveness of the six simulated calls in eliciting responses, as shown in Figure 4.21, and these differences were significant (Kruskal-Wallis ANOVA $H = 74.032$, d.f. = 6, $p < 0.001$). There were more responses in the silence period compared to stimulus B. It may have been that some of these responses were as a result of a latent response. Of the 33 responses that occurred when the Autobat was silent, 20 of these occurred within the first 30 seconds of silence, which suggests there may have been latency in responses to the previous stimulus broadcast. Table 4.6 details the post hoc analysis (using Mann-Whitney U with a *Bonferroni* correction to reduce Type 1 error rate (.05/7) resulting in a critical value for significance of 0.007) to investigate where the differences between call types were significant. Calls A & D (both high intensity with a high repetition rate) elicited a significantly higher response rate than any of the other call types, but there were no significant differences between call A & D or between the effectiveness of any of the other call types. A comparison of responses by known females (see Section 4.4.3.2) to different call types was compared to the responses of unknown individuals to determine whether the known females were more likely to respond to specific call types. There was no significant difference between groups (Kruskal-Wallis ANOVA $H = 2.997$, d.f. = 1, $p = 0.08$).

4.5.3.8 - Seasonal variation in stimuli effectiveness

There was evidence of an interaction between season and call effectiveness, as shown by the results of the Scheirer-Ray-Hare test detailed in Table 4.7. A total of 595 post-hoc pair-wise comparisons were undertaken (using Mann-Whitney U with a Bonferroni correction to reduce Type 1 error rate (.05/35) resulting in a critical value for significance of 0.0014) to determine whether the differences were significant. Figure 4.22 shows the results of the mean number of responses for each call type in each month. There was evidence of a seasonal difference in call effectiveness whereby in May, calls A & D (high intensity high repetition) were significantly more effective than call C (low intensity high repetition) but not significantly more effective than any other call type. In June, calls A and D were significantly more effective at eliciting behavioural responses than any of the other four calls. In July, call A was significantly more effective than C (but call A was not significantly more effective than any other call type) and D was significantly more effective than calls B (low intensity low repetition) and C. In August, there were no significant differences in the effectiveness of the 6 different calls. In September, call C was significantly more effective in eliciting responses than call B (but call C was not more effective than any other call type). The response rates for silence in August and September were high in comparison to May to July responses rates for silence and higher than to two of the calls in August (although these differences were not significant). This could be as a result of the latency of response for juveniles.

4.5.3.9 – Stimuli amplification

There was no significant difference in the number of responses per hour when responding to amplified and non–amplified calls (Mann-Whitney U = -0.080, n = 348, p = 0.936). The effect of amplification was analysed separately for each of the six social calls, as shown in Table 4.8, but there were no significant differences in the monthly number of responses for each call type between amplified and non-amplified calls. There was a significant association between amplified calls and proximity of overall approach. Close approaches were more frequently observed without the stimuli amplification whereas, in comparison, the distant approaches occurred more frequently when the stimuli were amplified (χ^2 7.54, d.f. 1, p = 0.006).

4.5.3.10 – Association between stimuli and behavioural categories

A highly significant association was found between stimuli type and behavioural category (χ_2 292.357, d.f. 25, p > 0.0001). However, this association test combined all calls and behavioural responses and did not provide any information as to which calls, if any, were more frequently associated with particular behavioural categories. Therefore, post-hoc analysis was carried out using cross tabulation and converting standardised residuals, the differences between observed and expected values, to a 'Z' score which, in turn, were compared to a critical value for alpha. Using a conservative critical value of Z = +/- 3.22 (corresponding to a p value of .0013, using a Bonferroni correction of .05/36 for comparing multiple comparisons), Table 4.10 shows the results of the post hoc analysis of the chi sq test of association. A total of three positive associations and one negative association were

significantly greater than the critical value of Z = 3.22 p = 0.0013. Call A elicited responses assigned to category five, call B elicited responses assigned to category one, and call C elicited responses assigned to category six, all more frequently than expected. By contrast, call D, was less frequently associated with responses assigned to category three than expected.

4.5.3.11 - P. auritus vocal responses

While filming the responses, a total of 481 sequences of vocalisations of *Plecotus auritus* were recorded. These included 251 recordings of echolocation calls only, 269 recordings that were a mixture of echolocation and social call, and 32 recordings that consisted of one or more social calls with no echolocation. A total of 348 *P. auritus* behavioural responses were captured on video whilst the Autobat was broadcasting stimuli. Of the 301 recordings that contained social call elements, 179 of the sequences were recorded whilst the Autobat was broadcasting the stimuli calls, and corresponded to the time when one (or more) *P. auritus* responded on video. There was a significant association between social call production and stimulus broadcast (χ_2 274.32, d.f. 1, $p < 0.001$). However, there was no evidence that any one of the six stimuli (A – F) was more likely to elicit a social vocal response over another call type (χ_2 8.41, d.f. 5, $p = 0.134$).

4.5.4 - Discussion

Behaviour can be viewed as a stream of elements, which, once accurately described, can be counted and timed (Donat, 1991). In order to record patterns of behaviour in a quantitative

manner, they must be broken down into identifiable and mutually exclusive categories that can be accurately assigned. One way of doing this is to use a dichotomous key. The most familiar use of dichotomous keys in biological sciences is in systematics (Metcalf, 1954) but they have also been used in animal behaviour research, for example in studies of hermit crabs *Calcinus tibicen* (Hazlett, 1980) and, also, in studies of mammals such as the common palm civet, *Paradoxurus hermaphrodites* (Krishnakumar et al., 2002), as an identification key to behaviours as it can be faster (and less ambiguous) to use than a list of descriptive behaviours.

The categorisation of a comprehensive description of behaviours for a species is defined as an 'ethogram' (Brown, 1975), which is the result of refining a catalogue of behaviours after many hours of observation and description (Lehner, 1995). The classification of behaviour of *P. auritus* in this study was based on their response to the stimulus, but does not in any way represent a complete repertoire of *P. auritus* behaviours. The classification of the behavioural responses of *P. auritus* to simulated social calls identified a number of different behavioural categories and allocation of behavioural responses to these categories was repeatable, as indicated by the inter-observer reliability tests. The categories differed predominately in observed physical behaviour, proximity of approach, and length of response. One potential disadvantage of categorising behaviours by human eye, however, is that the threshold values are not clearly defined and a small parameter relevant to the animal could be missed by the human (Martin and Bateson, 2007).

Categorisation of behavioural responses to artificial stimuli has been less reported than behavioural observations, but a recent study using video playback investigating the responses of domestic dogs to a robotic model dog with differing tail conditions (short/still, short/wagging, long/still, long/wagging) found that the behavioural responses of the approaching dogs could be categorised and differing behaviours were observed depending on the stimuli used (i.e. long wagging tails were significantly more likely to elicit an approach response compared to sort still tails) (Leaver and Reimchen, 2008).

The behavioural responses of *P. auritus* to the Autobat stimulus were assigned to six main categories within which further sub-categories were defined depending upon how close the responding bat approached the stimulus and which combination of behaviours were subsequently observed in proximity to the stimulus. The behavioural responses of *P. auritus* were frequently characterised by hovering (pause in flight to stay suspended in the air, fluttering in one place) adjacent to the stimulus. Hovering flight is a common habit of *P. auritus* (Norberg, 1976). Hovering was observed in over 53% of the behavioural responses of *P. auritus* whereas it was not observed in the behavioural responses of any other species. Hovering was observed in both short duration responses (category six) for *P. auritus* and, also, in longer, more complex responses (categories three and five). This interspecific difference in response categories is not as a result of hovering being a unique behaviour to *P. auritus*, as hovering has also been reported for *M. nattereri* (Swift and Racey, 2002).

The number of behavioural responses to the stimuli varied seasonally. In May, not only were the responses observed short duration responses (categories one and two), there were also significantly fewer responses to the stimuli. In June responses that were more complex and longer in duration occurred significantly more frequently. Later in the season there were no

significant differences in response categories but, overall, there were fewer *P. auritus* responses recorded to the stimulus in September, compared to August. The variation in the number of responses and types of responses recorded may be as a result of reproduction. For, example the energy demands for *P. auritus* females is greatest in June and July when gestation occurs. Evidence also suggests that offspring, once volant, share feeding areas (Chapter 7).

There are likely to be many factors that influence the nature or strength of an individual's response to stimuli, such as stage in the breeding cycle, presence or absence of offspring or other conspecific, or, potentially, the position of the stimulus within a feeding range of an individual. Female Northern bats *Eptesicus nilssoni*, for example, forage in small feeding areas that are used on consecutive nights. Reproducing females defend feeding territories against other colony members as well as non-members by means of aggressive chases and vocalisations. Intrusions into occupied feeding sites resulted in territorial conflicts in 47% of cases, passive departure by one or more opponents in 43%, or mutual acceptance in 11% of cases. Conflicts occurred regularly throughout the summer, but became less frequent in July when insect abundance increased (Rydell, 1986). In the mating seasons male bats, such as pipistrelle species (Lundberg and Gerell, 1986) and sac-winged bats *Saccopteryx bilineata* (Heckel and von Helversen, 2002), display territorial tendencies, characterised by chasing and vocalising at male intruders.

In this study, the sex of the responding individual was not known for most of the individuals responding to the stimulus, and this prevented conclusions being drawn as to why specific individuals may have responded in such a manner at that time. However, when the subset of the responses for known females was compared to the rest of the responses, there was no

apparent difference in the composition of response type between the groups. If the rest of the responding bats included lots of male responses, then it may be expected to find differences in the composition of responses or, conversely, if only females hovered then it would be expected to find a higher proportion of hovering in the known female subset of responses. No hovering was observed in the male bats marked with reflective bands. However, this sample size was too small to be conclusive.

There were also significant overall differences in the effectiveness of calls of different intensities whereby loud calls with high repetition rates (calls A & D) elicited significantly more responses than quiet calls. This may be explained by that fact that loud, more repetitive calls could potentially be heard by more bats which, in turn, could elicit a greater number of responses. However, if this were true, it would be expected that amplification of these calls would further increase the number of responses obtained but, in this study, no such effect was apparent. This may be because only individuals that regularly utilised the area, near to which the stimulus calls were broadcast, may have been motivated to respond to the stimulus whereas, individuals further away that may have heard the amplified call where less likely to alter their behaviour. In many species, individuals compete for resources but avoid escalated conflicts by using threat displays that allow the opponent to predict the outcome of the conflict without the necessity of fighting (Bradbury and Vehrencamp, 1998; Maynard-Smith and Harper, 2003). A study of male sac-winged bats *S. bilineata* found that male territorial holders can adjust their vocal territorial displays according to the peak frequency of the territorial songs of their opponents (Behr et al., 2009). Playback experiments with territorial males found that low frequency stimuli elicited a higher territorial song rate and length than high frequency stimuli. It has been shown that male *S. bilineata* that sing more often with lower peak frequencies sire more offspring than their competitors (Behr et al., 2006), and the

peak frequency of territorial calls may indicate male quality and the resulting threat posed to competitors.

Loud, long-distance vocal signals can serve a number of different functions simultaneously. Song in male birds, for example, not only functions in territorial defence, but is also used to attract mates and can advance female ovulation (Catchpole and Slater, 1995). Similarly, some male mammals have loud calls that serve multiple functions within the context of reproduction and indeed these functions can be entirely compatible if the signaller is both ready to engage in male–male competition and to mate with females (e.g. red deer *Cervus elaphus*) (Clutton-Brock and Albon, 1979). In this study it may be that the observed increased response rate for high intensity high frequency calls is a result of a number of different causal factors, which may interact in unison.

There was also evidence of seasonal variation in the response to each call type. Earlier in the season loud, high repetition rate calls were significantly more effective whereas later in the season quiet calls were just as likely to elicit an approach response. In turn, later in the season, quiet, high repetition rate calls were significantly more likely to elicit an approach response than quiet low calls. Many studies have found differences in social call production that relates to mating. For example, studies of the social calls of the common pipistrelle bat *Pipistrellus pipistrellus* found that the production of songflight calls occurred more frequently in the mating season (August – September) (Russ et al., 2003; Budenz et al., 2009), and social calls recorded at hibernation sites of greater horseshoe bats *Rhinolophus ferrumequinum* in late October were inferred by the authors to be mating calls, as these calls were absent from summer roosting sites (Andrews et al., 2006). This assumption, however, is speculative as social calls produced at hibernacula may have other functions such as information transfer (Wilkinson, 1995). To date no studies have investigated the effects of

season on the responses to social calls of differing intensity. This study has demonstrated that loud, repetitive calls elicit stronger responses earlier in the season compared to later on in the season, which could mean that loud repetitive calls could be used as defence of resources at a critical time early in the season or for attracting conspecifics to a rich resource to enable exploitation.

In addition to the effect of seasonal differences in responses in relation to call intensity, there was also evidence of an association between call type and observed behaviour. Loud calls with high repetition calls (such as call A) were significantly more frequently associated with a response assigned to behaviour categories that were longer in duration and more complex, whereas quiet calls (such as B and C) were more frequently associated with shorter duration responses. However, it is not possible to deduce from these patterns the functions of these differing intensity calls types as, in this scenario, the identity of the responding individuals was not known in most instances. There was no significant difference in the composition of responses to different call types between known females and the remaining responses where the sex of the bat was not know. However, the general trend does allow for the formulation of hypotheses, for example, do high intensity calls elicit more prolonged responses for females earlier in the season as a function of resource defence? This hypothesis could subsequently be tested under different conditions, such as a smaller woodland with all of the females radio-tagged and marked with reflective tape.

Social calls were recorded at the same time as 51% of the filmed behavioural responses of *P. auritus*. Although there is the possibility that these call(s) were not produced by the responding bat(s), this is extremely unlikely given that *P. auritus* social calls are recorded so infrequently under natural conditions. This supports the hypothesis that responses to the

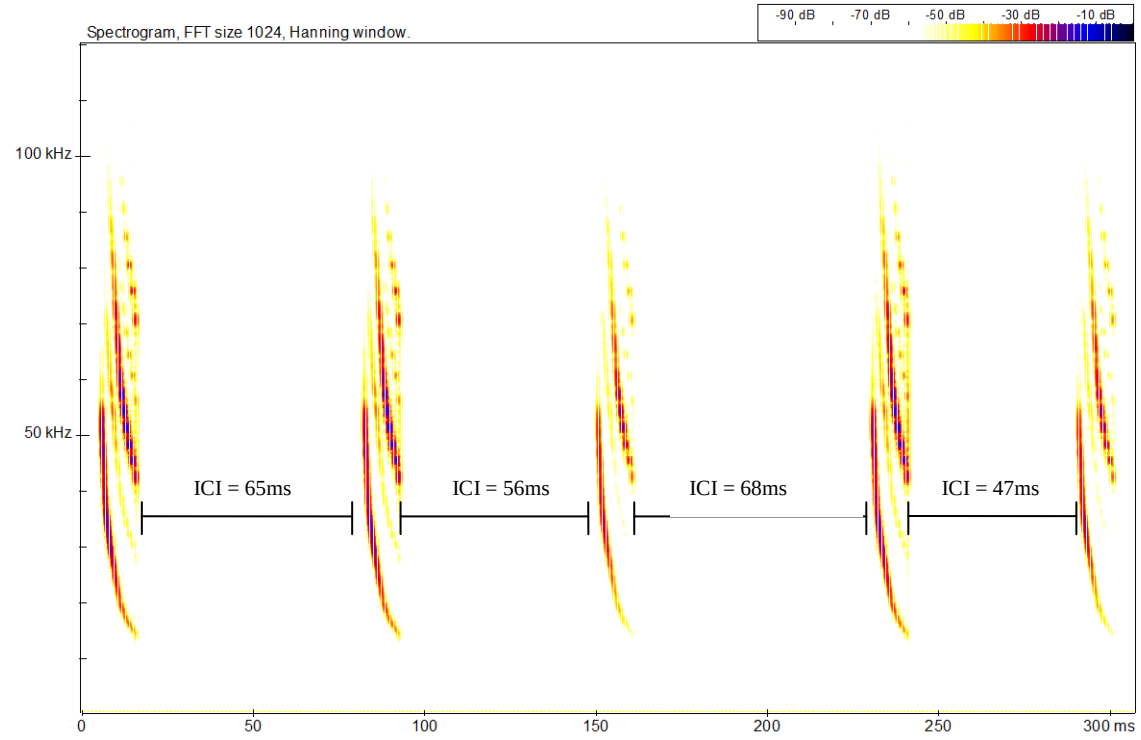
stimulus represent a social response as opposed to simply an investigation of a novel sound source. Social call production was significantly associated with the production of the stimulus which suggests that the bats were vocally responding to the stimulus. Many bat species use specific vocalisations in a given behavioural situation (Barclay and Thomas, 1979; Pfalzer and Kusch, 2003; Davidson and Wilkinson, 2004), and these vocalisations have been shown to convey information about both individual (Fenton et al., 2004) and group identity (Boughman and Wilkinson, 1998). A perception of identity (Balcombe and McCracken, 1992; deFanis and Jones, 1996) and group association (Boughman and Wilkinson, 1998) has, in addition, been reported for some bat species.

4.6 - General Discussion

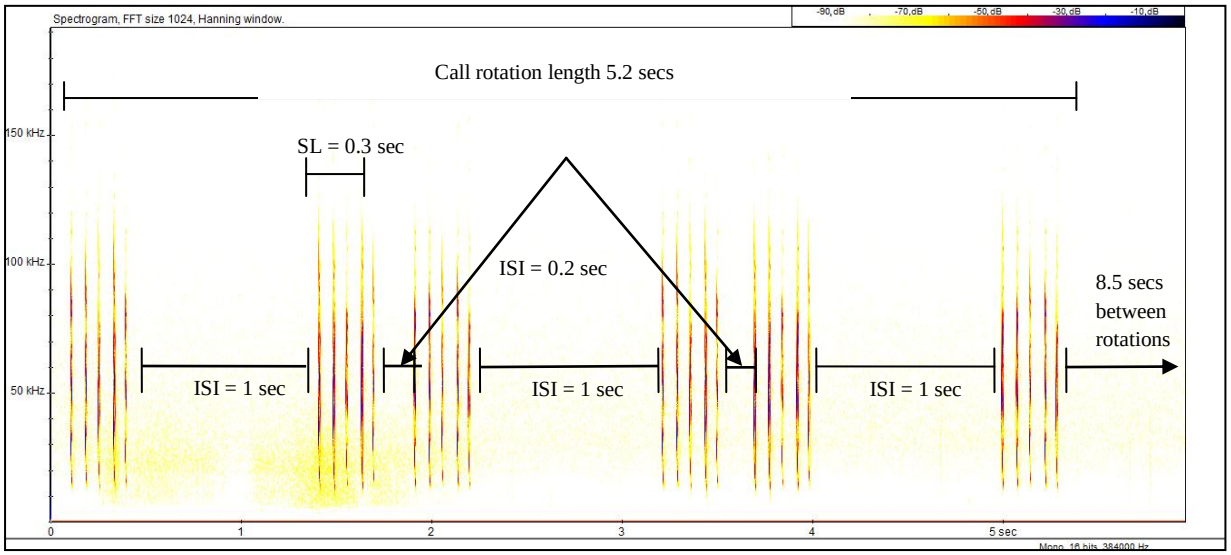
One explanation for the females' response to the Autobat may be that it represents an investigation of, and an attempt to expel, an unfamiliar bat detected within the home range. Defence of a feeding area may be more critical during late pregnancy and lactation, when energy demands on the females are highest. This may help to explain why female responses (at least as reflected by capture rates) declined in the latter half of the summer. One way of investigating a possible home range defence role of responses to social calls is to compare the responsiveness of females in relation to whether the stimulus is located within or outside of their ranging area. However, to determine whether the context the call was produced in is important, knowledge pertaining to the home range of the focal individual, and the distance that they are likely to respond to the stimuli, must be undertaken.

4.7 - Summary

- There were significantly more responses filmed, per hour, when the Autobat was broadcasting social calls than when the Autobat was silent.
- *P. auritus* were clearly much more responsive to simulations of their own species' social calls than to the other stimuli tested
- This chapter has shown that responses vary in relation to differences in the stimulus, and, also, seasonally. Although responses by banded individuals were too few to allow a detailed analysis of sex differences in response, the seasonal change in captures suggests that the function of the calls varies seasonally, with males becoming more responsive as the mating season begins.



Sonogram 1



Sonogram 2

Figure 4.1: Simulated *P. auritus* social call, 'stimulus A' was comprised of a sequence of five descending FM sweeps as shown in sonogram 1. These were played as a series of sequences at irregular intervals as shown in sonogram 2. The entire length of the stimulus rotation is 5.2 seconds. This was followed by an interval of 8.5 seconds of silence before the stimulus rotation was repeated again. ICI = inter-call interval (time). ISI = inter-sequence interval (time). SL = sequence length (time).

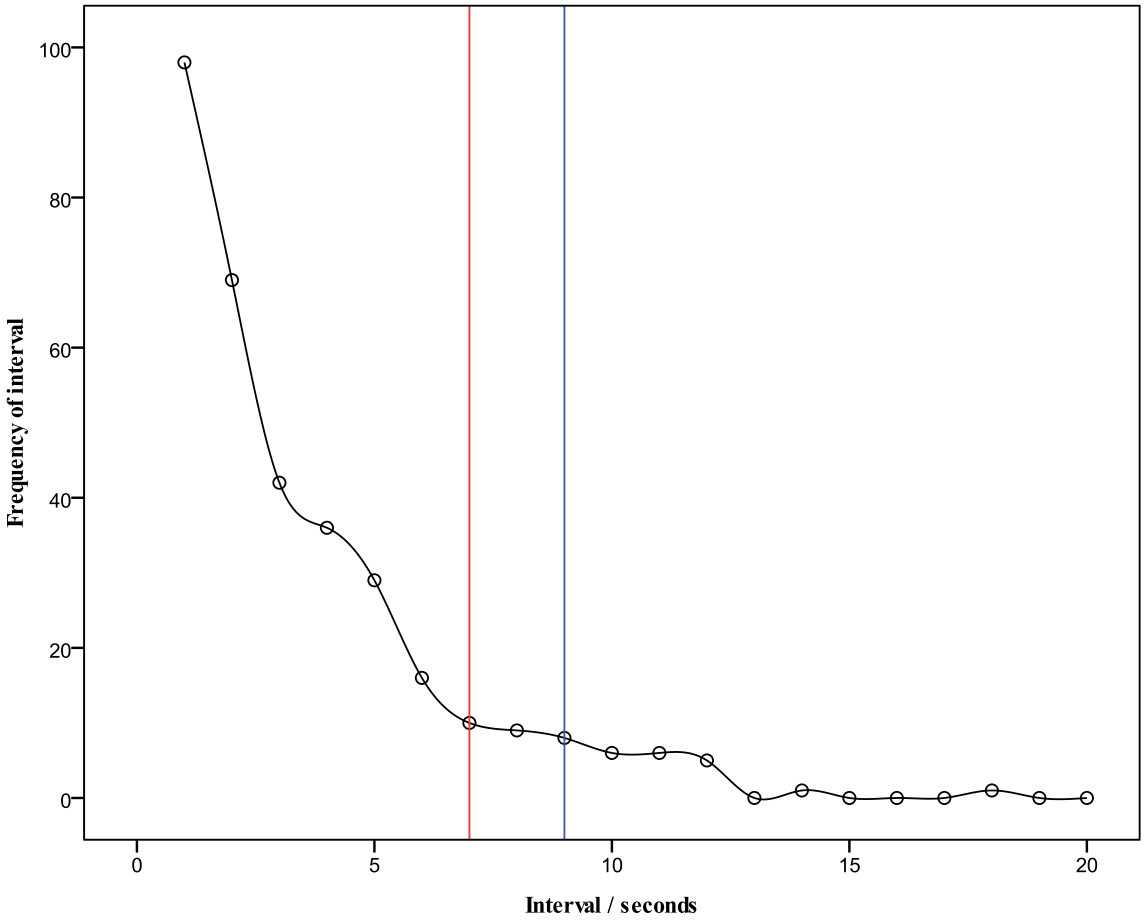


Figure 4.2: The frequency of observations of bats recorded on video was plotted against the interval between successive observations in order to obtain a suitable interval between observations to edit the film into separate 'responses'.

Figure 4.3: This photograph shows that the bat was within 12.5 cm of the box in the vertical plane, but it is not possible to tell how far it was in the horizontal plane.

Figure 4.4: This photograph shows that the same bat was within 25 cm of the box in the horizontal plane.

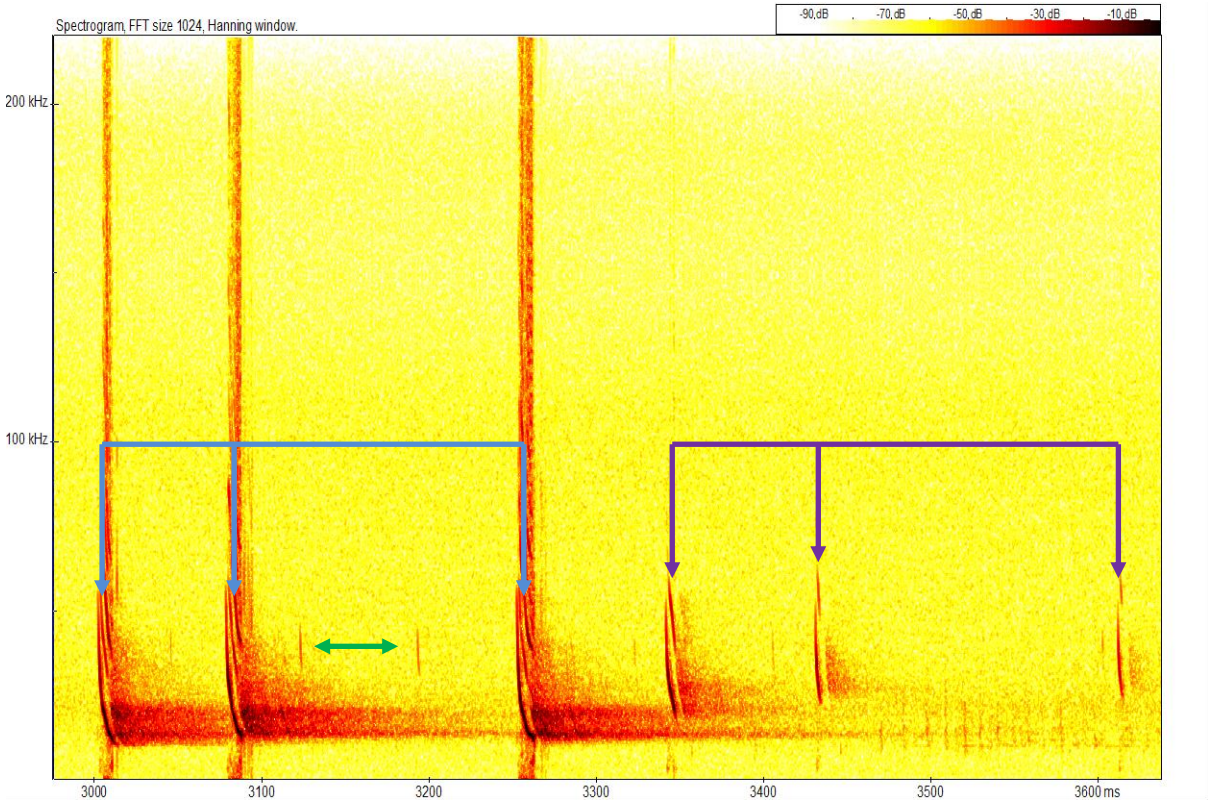


Figure 4.7: A *Plecotus auritus* response to synthesised social calls produced by the Autobat. The simulated social calls are indicated by the blue arrows. The responding *Plecotus auritus* initially echolocates between the simulation broadcast, as indicated by the green arrows, but three clear social calls are produced at the end of the sequence, as indicated by the purple arrows.

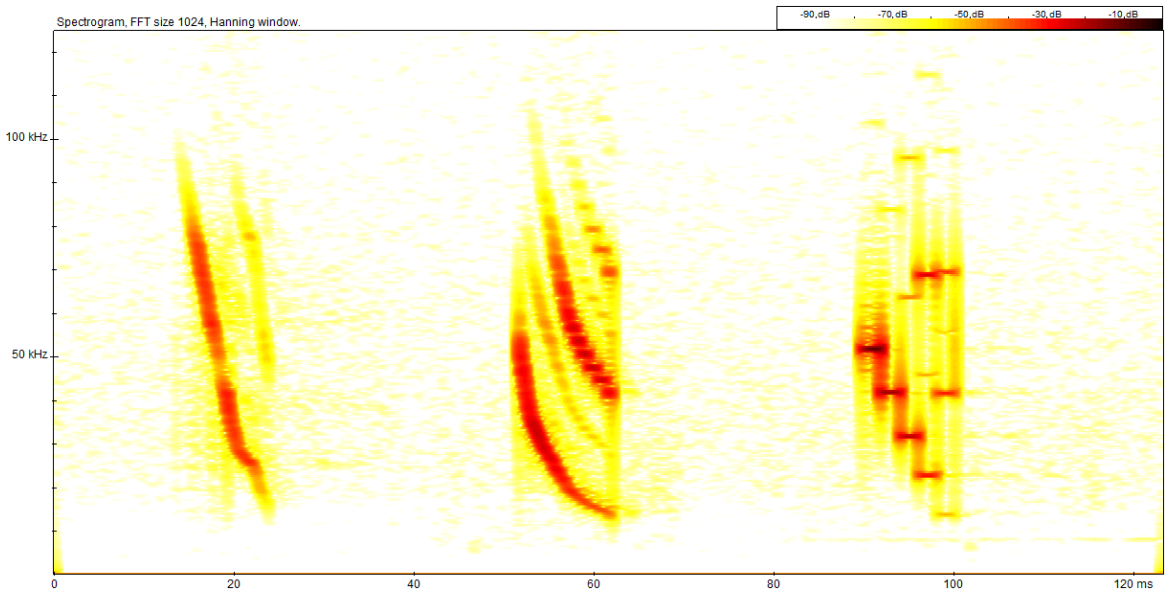
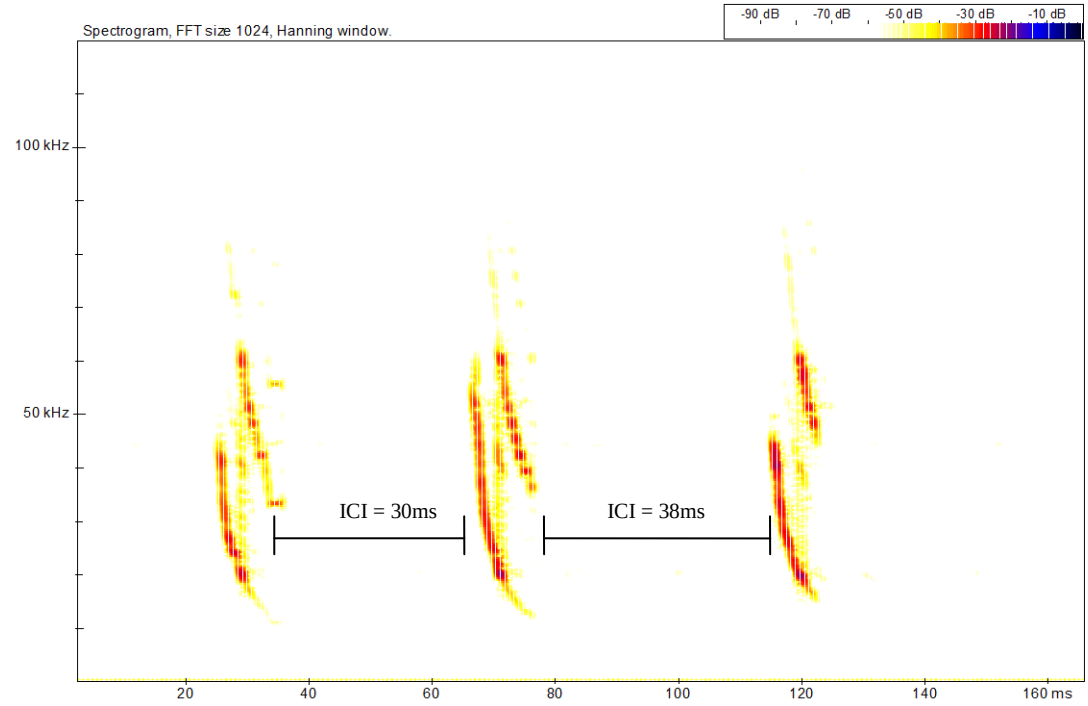
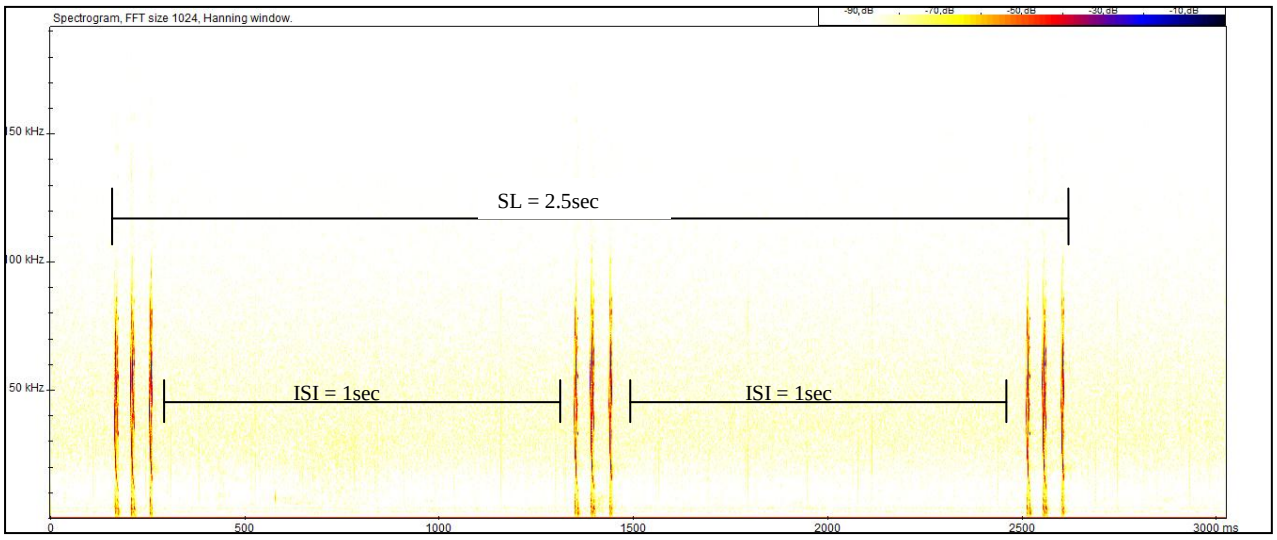


Figure 4.8: The first sweep is a simulated Bechstein's bat social call; the second is a simulated Brown long-eared bat social call and the third is a series of five brief, descending tones that follow a similar trajectory to the brown long-eared call. Each sweep is between 11 and 12 milliseconds in duration. Each was played in an irregular sequence of five sweeps, following the same temporal pattern for each call, with the same intersequence interval.

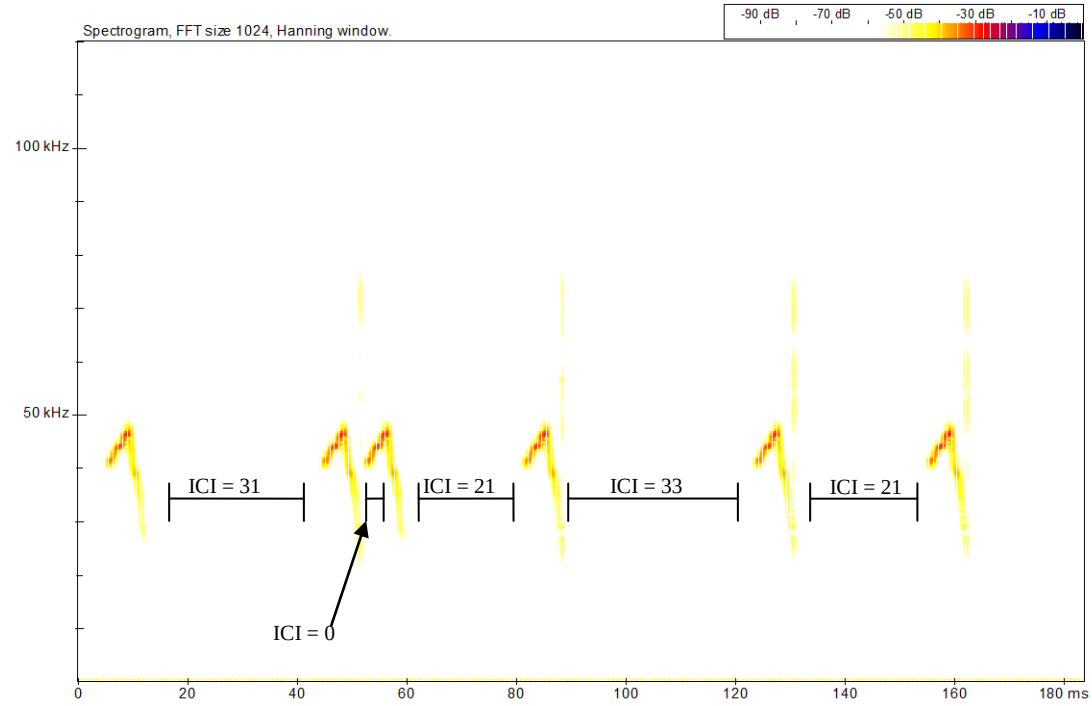


Sonogram 1

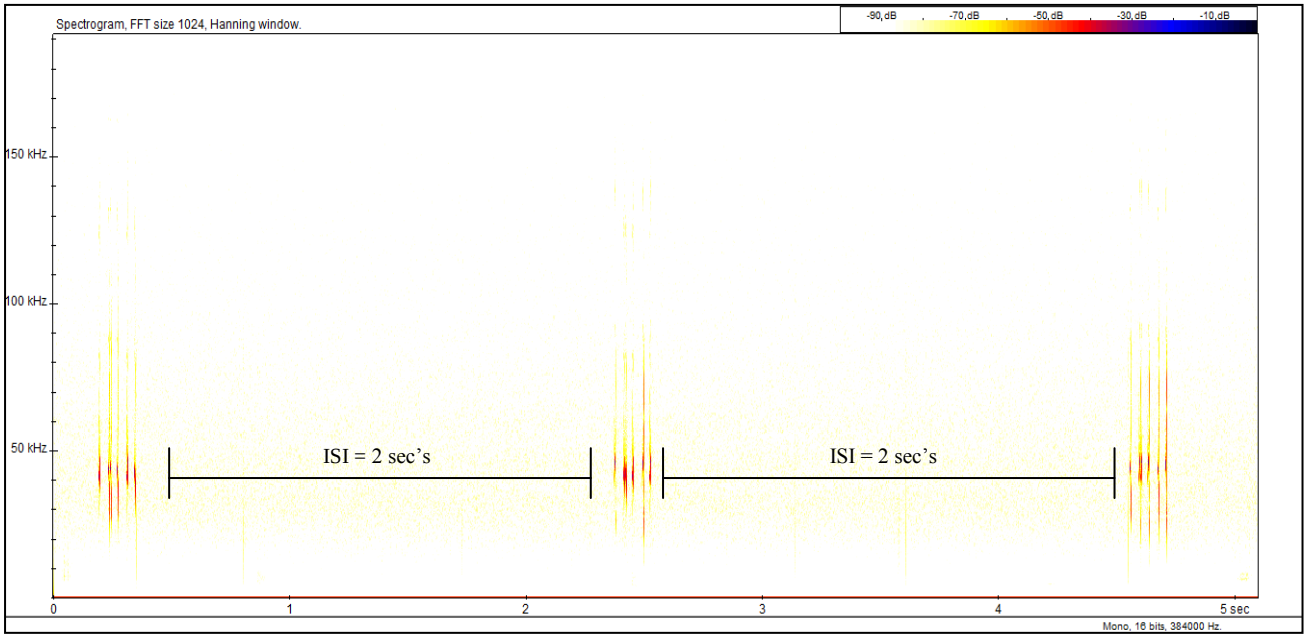


Sonogram 2

Figure 4.11: Simulated *Plecotus auritus* social call, 'stimulus B' was comprised of a sequence of three descending FM sweeps as shown in the sonogram 1. These three sweeps were played as a series of three sequences at one second intervals, as shown in Sonogram 2. The entire length of the stimulus rotation is 2.5 seconds. This was followed by an interval of 9.5 seconds of silence before the stimulus rotation was repeated again. ICI = inter-call interval (time). ISI = inter-sequence interval (time). SL = sequence length (time).

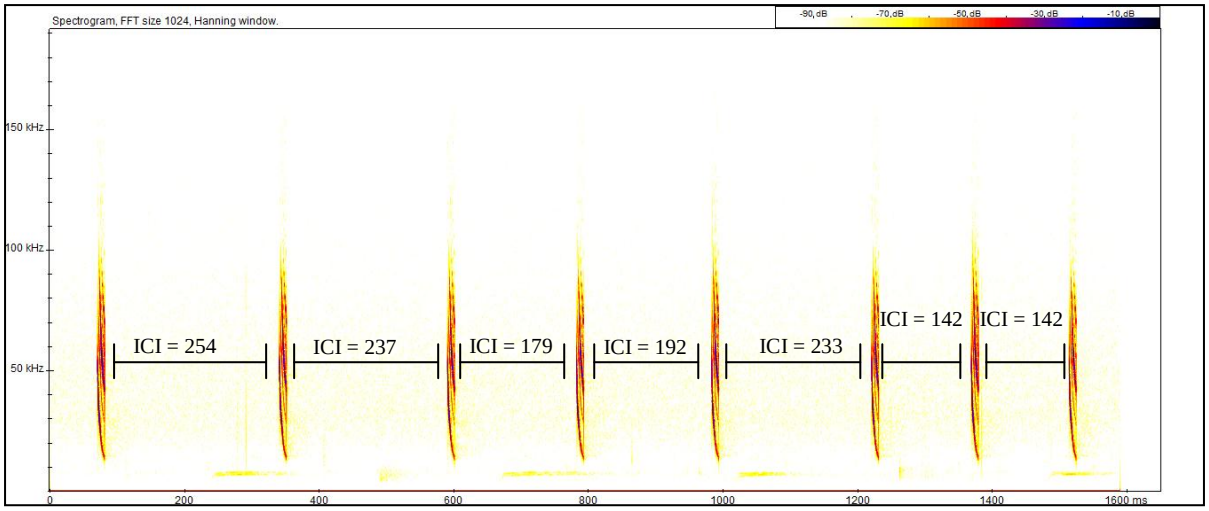


Sonogram 1

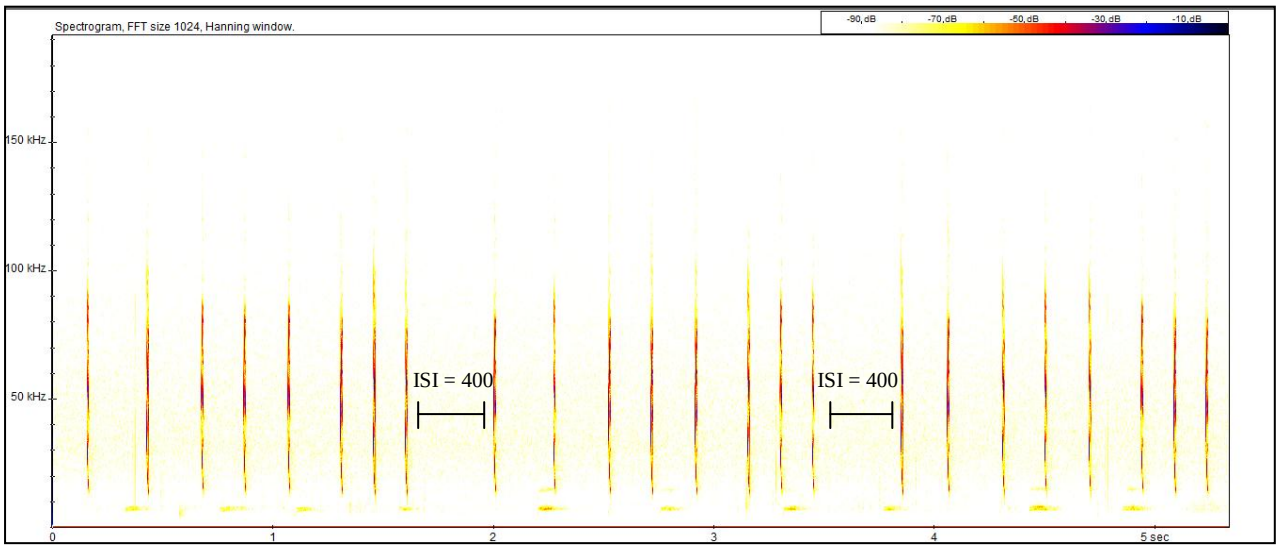


Sonogram 2

Figure 4.12: Simulated *Plecotus auritus* social call, 'stimulus C' was comprised of a six FM sweeps that ascended and descended shown in sonogram 1. The sequence lasted for a total of 160ms with an interval of two seconds between each sequence before the sequence is repeated again. ICI = intercall interval (time). ISI = inter-sequence interval (time).

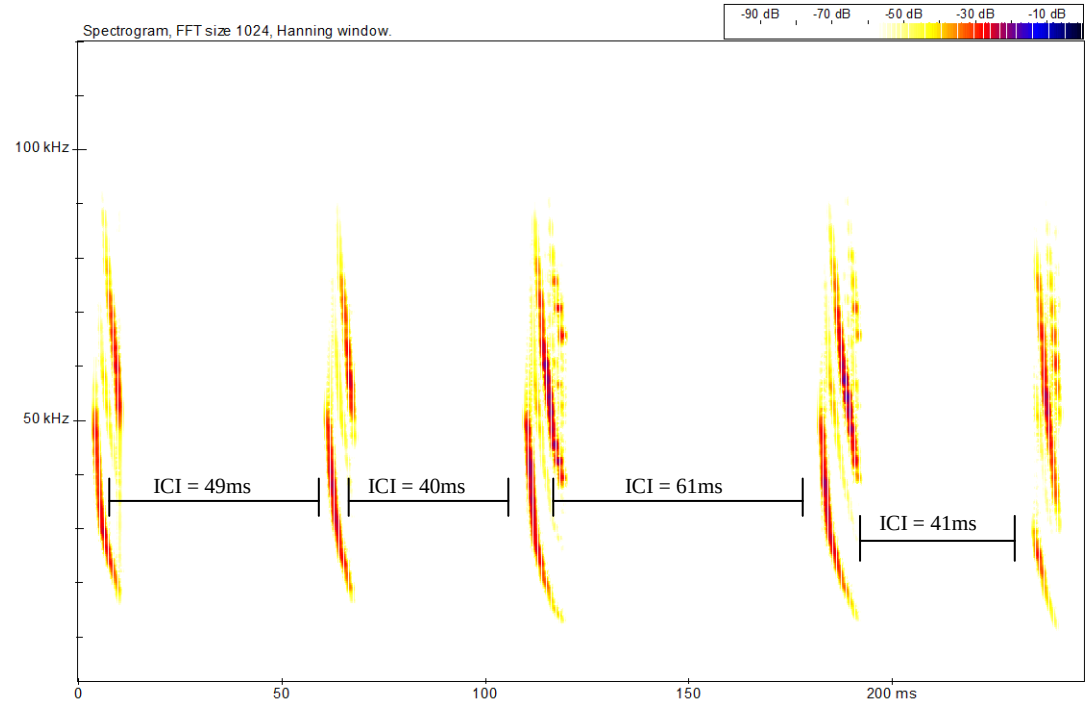


Sonogram 1

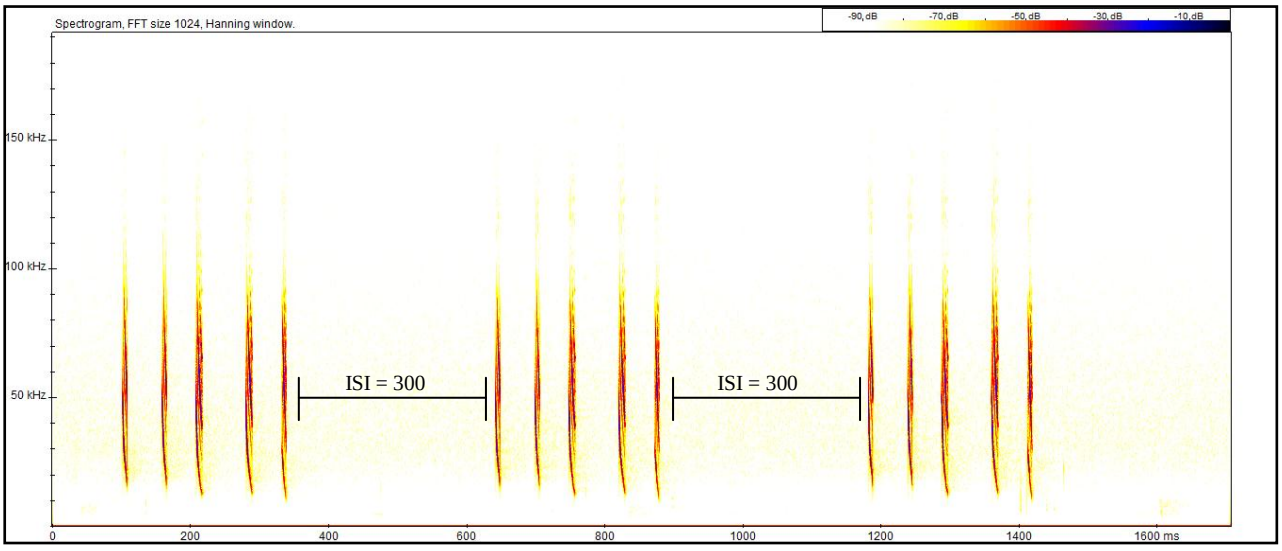


Sonogram 2

Figure 4.13: Simulated *Plecotus auritus* social call, 'stimulus D ' was comprised of a sequence of eight descending FM sweeps as shown in sonogram 1. These eight sweeps were played as a series of ten sequences, with an interval of 0.4 seconds, as shown in sonogram 2 (only three of the ten sequences shown). The entire length of the sequence rotation is 18 seconds. This was followed by an interval of 12.5 seconds of silence before the call rotation was repeated again. ICI = inter-call interval (time). ISI = inter-sequence interval (time). SL = sequence length (time).

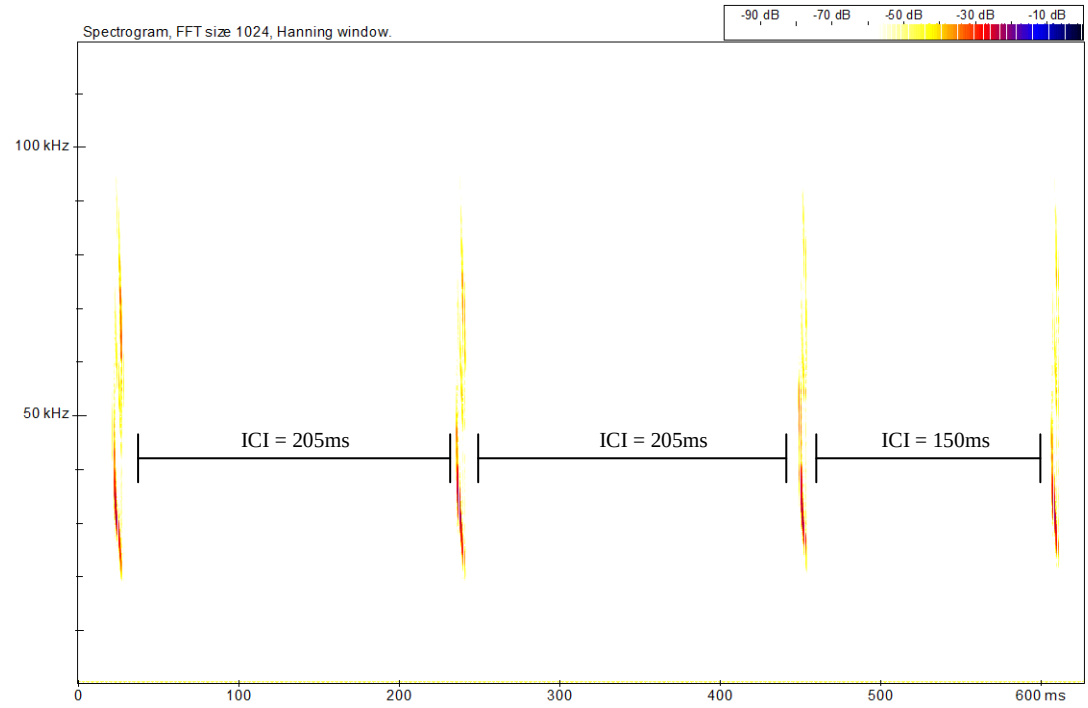


Sonogram 1

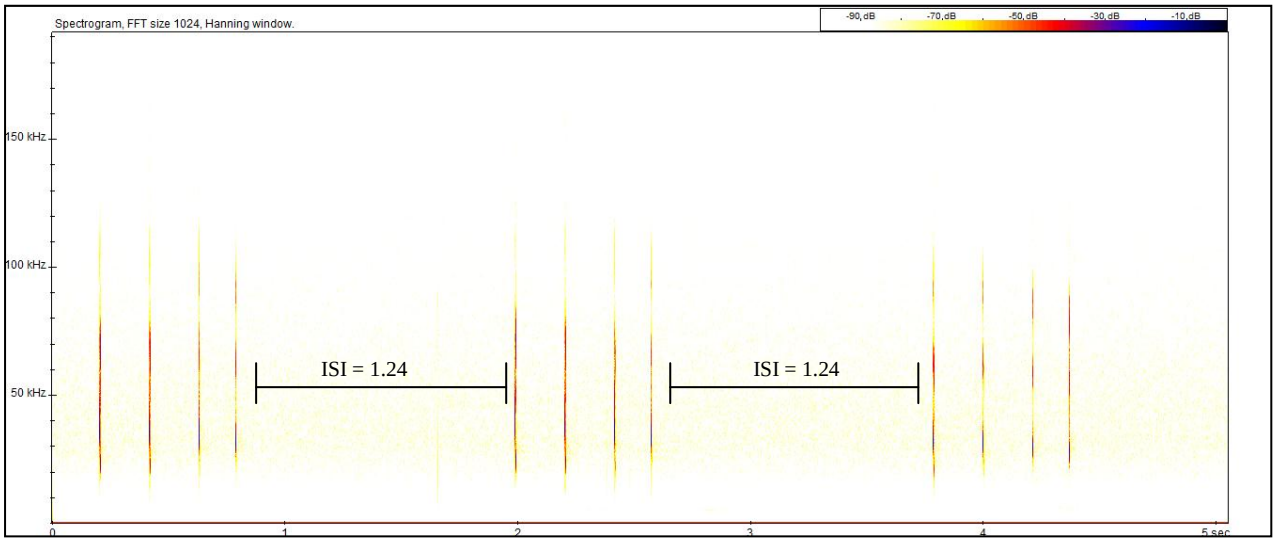


Sonogram 2

Figure 4.14: Simulated *Plecotus auritus* social call, 'stimulus E' was comprised of a sequence of five descending FM sweeps as shown in sonogram 1. These five sweeps were played as a series of three sequences at 0.13 second intervals. The entire length of the call rotation is 0.13 seconds. This was followed by an interval of 6.7 seconds of silence before the stimulus rotation was repeated again. ICI = inter-call interval (time). ISI = inter-sequence interval (time).



Sonogram 1



Sonogram 2

Figure 4.15: Simulated *Plecotus auritus* social call, 'stimulus F' was comprised of a basic sequence of four descending FM sweeps as shown in sonogram 1. These four sweeps were played as a series of nine sequences at intervals of 1.24 seconds, (three sequences are shown in sonogram 2). The entire length of the call rotation is 16.5 seconds. This was followed by an interval of 12.5 seconds of silence before the call rotation was repeated again. ICI = intercall interval (time). ISI = Intersequence interval (time = seconds).

	A	B	C	D	E	F
No of sweeps per sequence	5	3	6	8	5	4
No of sequences in rotation	6	3	n/a	10	3	9
No of sequences / min	26.28	15	27.77	19.67	7.47	18.63
No of sweeps / min	131.4	45	166.66	157.37	112	74.52
Number of sweep types / sequence	1	3	2	1	3	1
Sweep type 1: Duration(ms)	12	12	8	12	9.1	8
Sweep type 1: Fmin(kHz)	13.7	11.1	25	13.2	14	20.6
Sweep type 1: Fmax(kHz)	56.3	50.9	51.2	67	60	58.5
Sweep type 1: Fmax(e)(kHz)	32.7	20.7	47	48.0	34	32
Sweep type 1: dB SPL @ Fmax(e)	51.5	43.0	38.7	53.8	44.0	48.7
Sweep type 2: Duration(ms)	n/a	11.5	8.9	n/a	12	n/a
Sweep type 2: Fmin(kHz)	n/a	12	19.9	n/a	12.5	n/a
Sweep type 2: Fmax(kHz)	n/a	69	49.7	n/a	61	n/a
Sweep type 2: Fmax(e)(kHz)	n/a	20.7	44.9	n/a	42.0	n/a
Sweep type 2: dB SPL @ Fmax(e)	n/a	42.0	37.0	n/a	49.5	n/a
Sweep type 3: Duration(ms)	n/a	9.3	n/a	n/a	7.8	n/a
Sweep type 3: Fmin(kHz)	n/a	14.9	n/a	n/a	10	n/a
Sweep type 3: Fmax(kHz)	n/a	48.7	n/a	n/a	33.7	n/a
Sweep type 3: Fmax(e)(kHz)	n/a	20.1	n/a	n/a	25.0	n/a
Sweep type 3: dB SPL @ Fmax(e)	n/a	42.3	n/a	n/a	37.1	n/a

Table 4.3: Acoustic parameters of the six stimuli A – F. Stimuli A, D, & F has only one sweep type per sequence (hence only one set of measurements for each stimuli). Stimulus C had two different types of sweep per sequence and stimuli B & E had three different types of sweep per sequence.

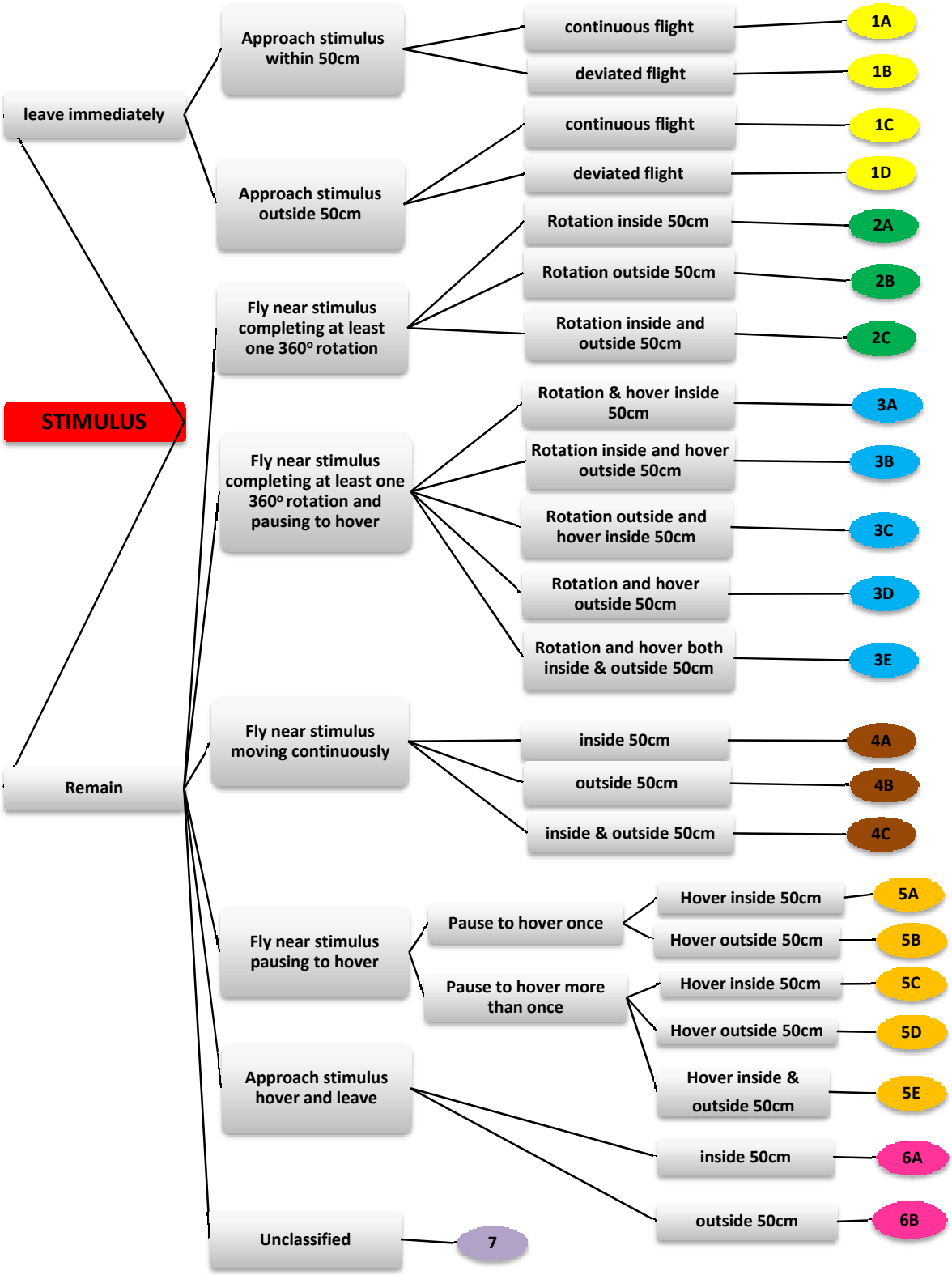


Figure 4.16: Categorisation of *Plecotus auritus* behavioural responses to simulated social calls.

Source of variation	SS	SS/MS total	d.f.	*P*-value
Month	34.438	23.58	4	0.0002
Response Type	59.573	40.80	6	0.0001
Month*Response Type interaction	88.600	63.28	24	0.0010

Table 4.5: The results of the Scheirer-Ray-Hare test showed that there are significant differences in relation to month, response type and a significant effect of an interaction for both month and response type.

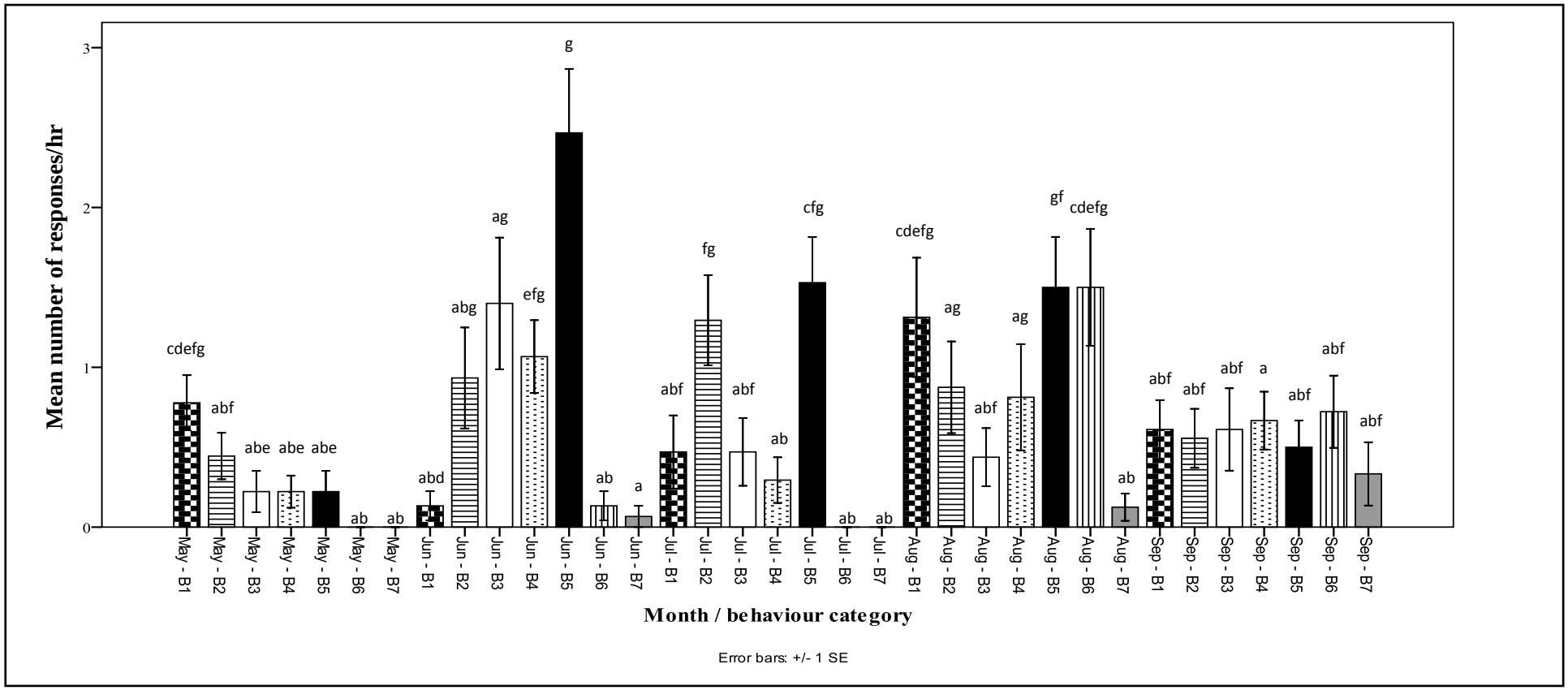


Figure 4.20: Mean number of responses for each behaviour category in each month. Where the letters are different it indicates a significant difference between groups.

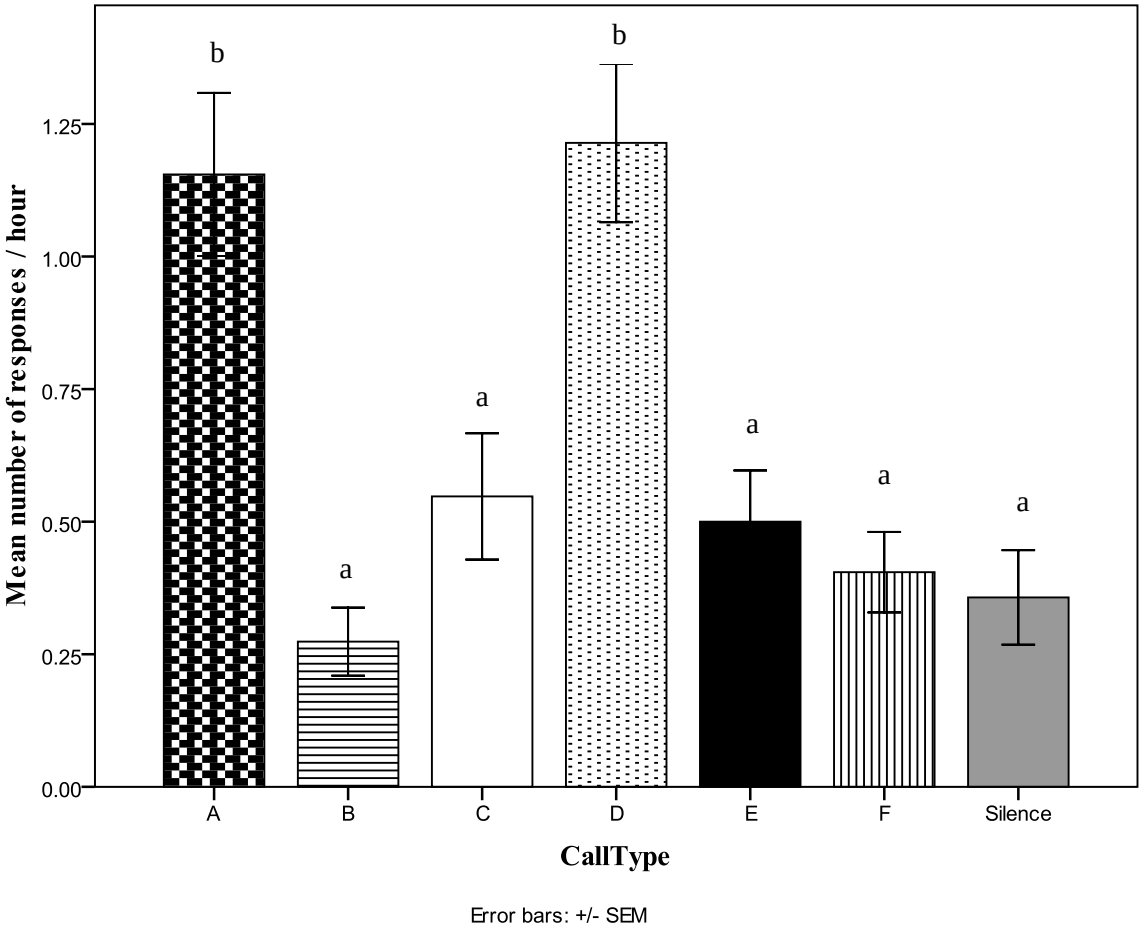


Figure 4.21: Total number of *P. auritus* responses to each stimuli type recorded over the 172 hours of filming. Both stimuli A and call D, which have a high intensity and repetition rate, elicited responses more frequently compared to, for example, call B, which has a low intensity and low repetition rate. Where the letters are different it indicates a significant difference between groups.

Source of variation	SS	SS/MS total	d.f.	*P*-value
Month	34.682	22.09	4	0.001
Call type	81.718	52.04	6	0.001
Month*Call Type interaction	99.266	63.47	24	0.001

Table 4.7: The results of the Scheirer-Ray-Hare test showed that there are significant differences in relation to month, call type and a significant effect of an interaction for both month and call type.

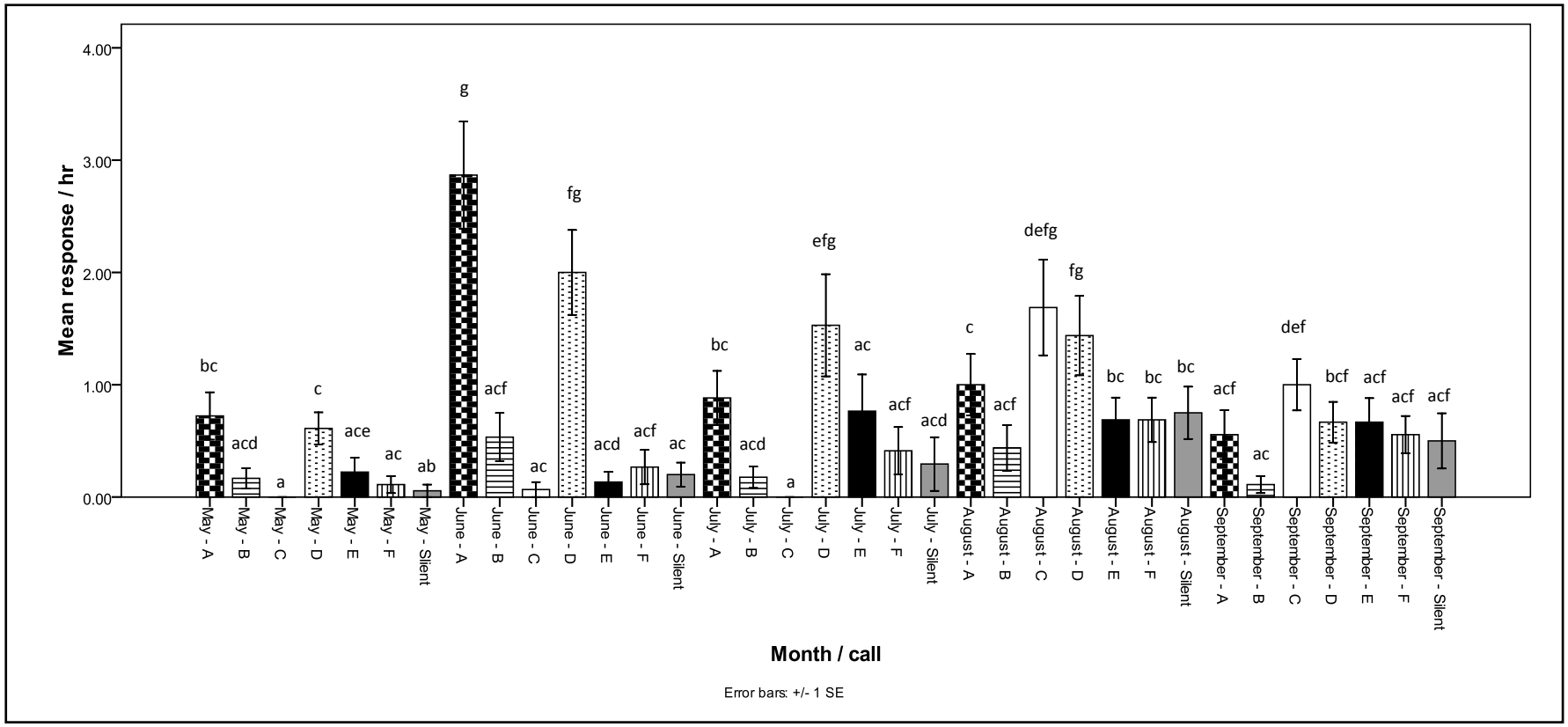


Figure 4.22: Mean number of responses for each call type in each month. Where the letters are different it indicates a significant difference between group means.

Call	**Test Statistic**	***n***	***p value***
A	Z = - 0.191	20	0.848
B	Z = - 0.191	20	0.848
C	Z = - 0.773	20	0.440
D	Z = - 0.038	20	0.969
E	Z = - 0.077	20	0.939
F	Z = - 0.824	20	0.410

Table 4.8: Effect of amplification on the number of responses for each simulated social call. There were no significant differences in the number of responses by amplification of each call type.

	No. of responses where approach was within 50cm of stimulus	**No. of responses where approach was outside 50cm of stimulus**	**Totals**
Call without amplification	133	38	171
Call with amplification	114	63	177
Totals	247	101	348

Table 4.9: There was a significant association between amplified calls and proximity of overall approach.

call * behaviour _category crosstabulation

			Behaviour category						
			1	2	3	4	5	6	Total
call	A	Count	6	14	21	11	45	1	98
		Expected Count	16.8	16.2	14.5	11.7	27.6	11.1	98
		Std. Residual	-2.6	-.6	1.7	-.2	**3.3**	-3.0	
	B	Count	12	6	2	5	0	0	25
		Expected Count	4.3	4.1	3.7	3.0	7.0	2.8	25
		Std. Residual	**3.7**	.9	-.9	1.2	-2.7	-1.7	
	C	Count	0	1	2	4	5	29	41
		Expected Count	7.0	6.8	6.1	4.9	11.6	4.6	41
		Std. Residual	-2.7	-2.2	-1.7	-.4	-1.9	**11.3**	
	D	Count	27	28	2	2	40	2	101
		Expected Count	17.3	16.7	15.0	12.0	28.5	11.5	101
		Std. Residual	2.3	2.8	**-3.4**	-2.9	2.2	-2.8	
	E	Count	10	2	15	14	5	2	48
		Expected Count	8.2	8.0	7.1	5.7	13.5	5.4	48
		Std. Residual	.6	-2.1	3.0	3.1	-2.3	-1.5	
	F	Count	4	6	9	5	2	5	31
		Expected Count	5.3	5.1	4.6	3.7	8.7	3.5	31
		Std. Residual	-.6	.4	2.1	.7	-2.3	.8	
Total		Count	59	57	51	41	97	39	344
		Expected Count	59	57	51	41	97	39	344

Table 4.10: Results of Chi Sq test of association. Standardised residuals that have a positive value mean that the cell was over-represented in the actual sample, compared to the expected frequency. Standardised residuals that have a negative value mean that the cell was under-represented in the actual sample, compared to the expected frequency. Using a critical value of Z = +/- 3.22, there are 3 positive (highlighted in yellow) and 1 negative (in red font) associations between call and observed behavior category.

5.1 – Introduction

Field experiments with infra-red video demonstrated that brown long-eared bats, *Plecotus auritus*, responded to synthesised social calls by rapidly approaching the source (Chapter 4). One hypothesis for the response of *P. auritus* to synthesised social calls at foraging sites is that the responses are related to home range use and represent defence of resources. In order to examine this hypothesis, it was necessary to establish the extent of home range and determine activity behavioural patterns.

'Telemetry' was defined by (Priede, 1992) as any method of obtaining information on living free-ranging animals by remote means. The attachment of radio-transmitters to animals allows the emitted signal to be located on subsequent occasions and, from this, the animal's movements can be inferred (Kenward, 1987). Radio-telemetry has become the method of choice for studying movement patterns and activity of many nocturnal mammals, including bats (Wilkinson and Bradbury, 1988). The benefits of radio-telemetry for studying bats include: (i) the locations of individual bats can be monitored over considerable distances, often for a period of weeks; (ii) direct observations of individuals during the day (i.e. day roost locations) and, potentially, during the night are possible because the bats location(s) can be determined (Wilkinson and Bradbury, 1988).

Most animals use the same area repeatedly over time (Darwin, 1861), hence animal movements are often defined using the home range concept (Jetz et al., 2004; Anich et al., 2009) where the home range is the area used by an animal over a given time interval (Burt, 1943; White and Garrott, 1990). Animals are expected to utilise home ranges that represent

the minimum economically defensible area (Pyke et al., 1977; Maher and Lott, 1995; Borger et al., 2008) but which are large enough to provide food resources and shelter over time (McNab, 1963; Reiss, 1988; Dammhahn and Kappeler, 2009). Some studies have proposed that the inter-specific variation in home range is typically correlated with body size (i.e. larger animals have larger home ranges) (McNab, 1963; Reiss, 1988; Dutoit, 1990). However, the size of an animal's home range may also vary with sex (Borger et al., 2006), season (Wiktander et al., 2001), age (Cederlund and Sand, 1994) and, also, population density (Damuth, 1981) both intra- and inter- specifically.

Radio-tracking studies of Microchiropteran bats have revealed inter- and intra-specific variation in home ranges. Range sizes for barbastelle *Barbastella barbastellus*, for example, have been reported to be in the region of 125 to 2551 ha (median:403ha for 12 radio-tracked females) (Hillen et al., 2009) whereas reported range sizes for Bechstein's *Myotis bechsteinii* females are substantially smaller, 9.9ha to 37.5ha (mean 20.4ha for 10 radio-tracked females (Kerth et al., 2001). Considerable variation in range size has also been reported for morphologically similar species. For example, radio-tracking studies on two species of sympatric pipistrelle bats in the UK *Pipistrellus pipistrellus* and *Pipistrellus pygmaeus* have revealed that *P.pipistrellus* home ranges were on average three times larger than those of *P. pygmaeus* (Nicholls and Racey, 2006) and there were also considerable differences in range size of individuals within species (Davidson-Watts and Jones, 2006). These reported variations in range sizes do not support the hypothesis that range size is related to body mass, and alternative explanations, such as the distribution of resources (e.g. prey) in a heterogeneous environment, may better explain variation in range size (Carr and Macdonald, 1986). Such patchy distribution of prey may mean that the resource defence hypothesis is not appropriate for some species of bat (Hickey and Fenton, 1990).

P. auritus is a gleaning bat (defined as capturing prey crawling or at rest on solid surfaces rather than in flight) (Swift and Racey, 1983; Anderson and Racey, 1993; Swift, 1998) that feeds predominately on Lepidoptera and Diptera (Swift and Racey, 1983; Rydell, 1989; Shiel et al., 1991; Vaughan, 1997). Detailed published information on *P. auritus* nocturnal behaviour is limited to one study in Germany which radio-tracked six females (all non-reproductive or lactating) (Fuhrmann and Seitz, 1992) and one published study in the UK which radio-tracked 16 bats over a total of 65 nights in north-east Scotland (10 female, all non-reproductive or lactating, and 6 males, all mature) (Entwistle et al., 1996). Both of these studies have indicated that this species forages preferentially in broadleaved deciduous woodland which concurs with the foraging preference for *P. auritus* in Southern England (Murphy et al. in prep). The radio-tracking studies in the UK have also demonstrated that female *P. auritus* rarely travel more than 2.5 km from the roost colony (Entwistle et al., 1996; Murphy et al., in prep) and have shown a high night to night consistency in the use of feeding sites (Entwistle et al., 1996). Having regular access to a suitable foraging area within this distance may be necessary for *P. auritus* to successfully raise young and repel intruders from foraging areas thereby reducing competition for resources within them.

This Chapter describes two studies. The first, detailed in Section 5.2 was a pilot study in which radio-tracking techniques were refined and suitable methodology for the analysis of radio-tracking data, in relation to home range use, was evaluated. The subsequent study, detailed in Section 5.3, ascertained nocturnal foraging behaviour, activity patterns and home range estimates for individual female *P. auritus* throughout the active foraging season (May – September), and identified key feeding localities for individuals.

5.2: Pilot Study

5.2.1 – Introduction

The aim of the pilot study was to assess suitable radio-tracking techniques and to review appropriate home range analysis methodology in order to design a subsequent radio-tracking study.

5.2.2 - Methods

5.2.2.1- Study area and capture of bats

Between 6 July and 30 September 2006 a total of six *P. auritus* (five female bats and one male bat) were radio-tracked at Plashett Wood, East Sussex (Grid Reference for centre of site OS TQ 546117 115659) and Ebernoe Common West Sussex (Grid Reference for centre of site OS SU 497745 127192). Bats were caught in either mist nets or harp traps, to which they were attracted using an acoustic lure (Sussex Autobat: Hill & Greenaway, 2005). Capture sites were located inside the wood away from edges and rides and trapping began approximately 60 minutes after sunset. This was done to maximise the chances of catching bats that were foraging rather than travelling to or from their roosts.

5.2.2.2 – Marking bats

Holohil LB-2 (Holohil Systems Ltd.) radio-transmitters were used for tagging the bats. The radio-transmitters weighed approximately 0.5 to 0.6 g including glue which represented approximately 5.9% – 7.1% of the bats weight (based on the mean weight range of the

captured bats). Transmitters were attached with Skin-Bond® (Pfizer Inc) to the area between the shoulder blades from which fur had been clipped. Recapture data indicates that the tags usually fall off the animals after 5 - 12 days. All animals were caught and handled under licence from Natural England. To determine the position of tagged bats during the night the animals were radio-tracked on foot using a Biotrack 'Sika' receiver and a Yagi 3-element antenna on a height-adjustable and portable mast.

5.2.2.3 - Radio-tracking technique and regime

A focal bat's position was determined by taking paired bearings sequentially from various known locations around the foraging area. Each location was 40 - 200m (depending on whether a triangulation could be obtained) from the next nearest location and could be reached by walking in less than one minute. Time, compass bearing, GPS reading and weather were recorded on data sheets in the field. The method of data collection was refined during the course of the study. Animals were radio-tracked from 1.5 hours after sunset (to exclude time spent commuting from their roosts to their foraging sites) for a period of 4 – 7 hours per night in July and August (mean = 4.5hr, SD = 1.08, n = 12) and for a period of 2 – 4 hours per night in September (mean = 3.16hr, SD = 1.32, n = 6). Only active fixes were recorded (to avoid biasing the dataset in favour of roost locations) and bats radio-tracked in September 2006 often returned to their roosts within 2 hours of emergence during adverse weather conditions (windy, heavy rain and low temperatures and less than 8^{o}C).

5.2.2.4 – Data analysis

The location and bearings were used to determine the bat's position using LOAS (version 4.0b, Ecological Software Solutions, 1998 - 2005). The estimated fixes obtained from LOAS

were subsequently imported to Biotas (version 1.03, Ecological Software Solutions, 1998 - 2003) producing a visual representation of the estimated foraging locations. The cumulative home range size was plotted against the number of successive locations for all bats radio-tracked in 2006 in order to determine the point at which the foraging area reached an asymptote, indicating that sufficient data had been gathered from each bat. Estimates of range size for this analysis were based on 100% minimum convex polygons.

5.2.2.5 - Home range analysis

The home range of an individual animal is typically constructed from a set of location points that had been collected over a period of time, identifying the position in space of an individual at many points in time. The concept of home range was described by (Burt, 1943) as 'the area, usually around a home site, over which the animal normally travels in search of food'. However, there is no standard definition of use and interpretations vary (Boulanger and White, 1990; Harris et al., 1990). Harris et al. (1990) argued that the choice of data to include in a home range estimate should depend on the aim of the analysis. If the aim is to detail the entire 24 hour period, then fixes should be collected evenly throughout this period. In these circumstances, many species ranges would, therefore, be centred on a lying up area (i.e. roost area for bats). However, if the aim is to describe the area used whilst foraging, then only active fixes should be considered for inclusion in the home range analysis.

A variety of analytical tools exist to estimate home ranges. These can be divided into two major classes: (i) minimum linkage approaches that describe ranges as polygons with minimised distances between edge locations; and (ii) probabilistic approaches that estimate the density of locations throughout a range (Harris et al., 1990; White and Garrott, 1990;

Kenward, 2001). Current analytical studies on animal home range use tend to employ both methodologies (Nicholls and Racey, 2006; Davison et al., 2009; Jhala et al., 2009). The Minimum Convex Polygon (MCP) (Mohr, 1947) is the oldest and most commonly used method of estimating home ranges and it facilitates comparisons with other studies. The peripheral locations of an animal are joined to each other using a connecting rule. The simplest and most popular connecting rule is simply to connect the outermost points on the scatter of mapped locations such that the sum of linkage distances between edge points is minimised. However, MCPs are very sensitive to outliers and require large data sets for accurate estimations of home range size (Powell, 2000). Furthermore, they give no information about how the animal is using its home range (Harris et al., 1990).

Probabilistic approaches to home ranges estimators have also been developed whereby the density of fixes is estimated throughout the area used by the animal. These approaches are less sensitive to sample size and outliers. Early methods estimated density as ellipses (Calhoun and Casby, 1958) by assuming that locations were distributed normally about one nucleus of activity. Ellipses do not define range shape well but require few locations to reach a maximum estimate and are, therefore, useful for identifying habitat available to animals that cannot be tracked frequently (Kenward et al., 2006).

The harmonic mean model (Dixon and Chapman, 1980) estimates the location density distribution (equivalent to the probability of encountering the animal) at intersections of an estimation matrix. Contours containing a specified percentage of actual locations or estimated location density are then interpolated across the matrix (Dixon and Chapman, 1980). Harmonic mean contours are sensitive to intersection spacing but provide contours least sensitive to outlying locations and are most precise in fitting core locations (Kenward et al.,

2006). This method also has the advantage that the boundary does not have to be elliptical (unlike a normal distribution) and it does not have to be one enclosed area (Harris et al., 1990). Harris et al., (1990) recommend using 80% isopleths, when the distribution of estimated locations are skewed and their number is low, to give a more accurate range representation (fewer than 150 fixes per sample is considered low; Kenward, 1992).

Kernel density estimation (KDE) (Silverman, 1986; Worton, 1987; Worton, 1989) is a non-parametric estimator that describes home ranges by means of hierarchical probabilities for the intensity of habitat utilisation, termed isopleths. The isopleths mainly depend on the probability distribution of single locations on an underlying two dimensional grid. A smoothing factor or bandwidth '*h*' defines the shape and width of this probability distribution. The resulting grid of summed influences represents the probability that an animal would be present at a certain point in its range at any given moment (Worton, 1987). Series of isopleths can be plotted around the smallest area where the cumulative probability reaches a particular value. For example, the 95% isopleth encompasses the area within the probability of finding an animal is 95%. KDE is mathematically robust and produces more consistent results than harmonic mean contouring, but is more sensitive to outlying locations (Kenward et al., 2006).

Taking these considerations into account, range analyses were conducted on location data that excluded commuting and 'inactive' fixes (i.e. periods of hanging up). Two home range estimators were used to calculate total home range for radio-tracked bats once the tracking period for each individual had finished: 100% minimum convex polygons and 80% isopleths harmonic mean. Once data had been collected from the first bat radio-tracked, a comparison of harmonic mean and fixed kernel analysis was carried out on the data points (fixed kernel

was initially chosen over adaptive kernel as data from only one individual existed at that stage). The comparative analysis, detailed in Figure 5.1, revealed that for this individual, kernel analysis better matched the distribution of the fixes. However, the resulting range estimates were very small and almost exclusively centred upon the fixes.

The harmonic mean method resulted in a larger range area which included habitat between fixes. A low number of fixes (40) was obtained for this female throughout the radio-tracking period (mean percentage contact time during radio-tracking was 87%) and, as a consequence, it was likely that kernel analysis would be not be appropriate to represent range use for this female. A study carried out by (Seaman et al., 1999) into the application of kernel methods used computer simulations of 10-200 points / home range to investigate the optimum number of observations that should be obtained for kernel estimates. Seaman *et al*. (1996) recommended that home range studies using kernel estimates should at least acquire a minimum of 30 observations per animal but preferably more than 50. Therefore, it was decided that the 80% HM isopleth would be adopted for this pilot experiment (given the likelihood that the number of fixes for each individual may be low). However, once the data had been collected from all radio-tracked bats in the pilot study, this was re-analysed using Kernel methods to ensure the appropriate home range analysis methodology was employed for the subsequent radio-tracking study detailed in Section 5.3.

5.2.2.6 - Core area analysis

Home ranges usually comprise heterogeneous areas determined by physical and biological factors which explain the disproportionate use of space by animals. Studies on various species show that, for a number of environment-related reasons, certain portions within the

home range are visited more frequently than others (Adams and Davis, 1967; Dixon and Chapman, 1980; Georgii, 1980; Macdonald and Courtenay, 1996). The centre(s) of activity can be defined as the area within the home range in which the most fixes occurred during the radio-tracking period and can give an indication of which part(s) of the range the bats used more intensively. Areas of more intensive use have been termed as the 'core area of the home range' of the animal and may be related to the greater availability of food resources and refuges (Samuel et al., 1985; Thompson et al., 2007).

Probabilistic home ranges (such as those derived from HM or KDE) allow identification of core areas by plotting the percentage of maximum probability of use (i.e. 10% isopleth, 20% isopleth etc.) on the x axis against the percentage of home range contained within the isopleth on the y axis (Harris et al., 1990; Powell, 2000). The resulting relationship shows: i) a descending straight line with a slope of -1 if space use is random; ii) a curve sagging below the line if space use is clumped; or iii) a curve above the line if space use is uniform. If a clumped relationship is shown, the core area can be identified at the point on the graph where the distance between the curve and the straight line is the greatest (Harris et al., 1990; Powell, 2000). The corresponding isopleth can be read off the graph. This analysis, termed 'utilisation plots' (as described by Powell, 2000), was used to assess whether bats had core areas that they use more intensively than other parts of their ranges. Core areas were identified by constructing utilisation plots, using figures derived from 80% Harmonic Mean analysis. This revealed that animals invariably showed clumping in their space use (Figure 5.2) with maximum clumping occurring between the 30% and 50% (Table 5.1). The median value of 40 % was adopted and applied to all analyses as a comparable estimate of core area.

5.2.3 - Results

5.2.3.1 - Ranging Area

Radio transmitters were attached to a total of six adult female bats and one adult male bat but data was obtained from only five of the six female bats (as one transmitter failed on *Plecotus auritus* before any data could be collected). Radio-tracking data from the first night after capture was not used in the analysis as a study on *Myotis bechsteinii* by (Fitzsimons et al., 2002) found that some tagged bats spent the night they were captured on their own, away from the main roost, which may have been as a direct result of being tagged. However, on the first night after capture, an approximation of the key foraging areas for each bat was determined which made subsequent nights radio-tracking easier. The six bats (5 female and 1 male) were radio-tracked for up to six hours per night for a total of 76 hours resulting in 311 estimated locations that were used for analysis. Data for two bats (one male, bleb 5, and one female, bleb 7) did not reach asymptote, as shown in Figure 5.3.

The 100% MCP and 80% HM home range estimators for all five bats radio-tracked at Plashett Wood were defined as shown in Figure 5.4 and Figure 5.5 respectively. Both the 100% MCP and 80% HM show that there was considerable overlap in home range amongst the radio-tracked individuals (however, as the individuals were not simultaneously radio-tracked, it is not possible to be conclusive about concurrently shared foraging areas). The 40% HM core foraging area for all five bats radio-tracked at Plashett Wood was defined, as shown in Figure 5.6, and there was overlap for two bats observed (however, again, this is not conclusive). The home range analysis for the bat radio-tracked at Ebernoe Common was defined as shown in Figure 5.7. This female had two clear foraging locations, one in Ebernoe

Common adjacent to her roost and a secondary foraging area in The Hoe approximately 1 km from her primary foraging area. The size of foraging area for each bat is detailed in Table 5.2. One bat (Bleb 7), had a home range (80% HM) which was 5 times greater in size compared to the mean of the other five bats and a core foraging area which was 4 times greater in size compared to the mean of the other bats. This bat was radio-tracked in poor weather conditions (very windy and high rainfall) and did not reach asymptote with respect to her foraging range size. She foraged predominately over hedgerows en route from her roost to Plashett Wood. Figure 5.8 shows the mean range size of all bats including and excluding this female.

5.2.3.2 - Ranging Analysis

At the end of the pilot experiment a re-analysis of all home range methodologies revealed that the use of harmonic mean methodologies produced large home range estimates and included substantial areas of habitat which were not visited by individuals. When the number of fixes exceeded 40, fixed kernel analysis more closely matched the distribution of the fixes for *P. auritus*, as shown by Bleb 4, Figure 5.9. The use of harmonic mean analysis for Bleb 7 (the female which did not reach the asymptote) resulted in a range that was vastly greater in size compared to the other females. This female foraged along hedgerows and a copse, as detailed by the estimates of foraging locations in Figure 5.10, and the resulting comparative analysis of HM and KDE, shown in Figure 5.11, revealed that KDE was more likely to be a more accurate representation of range use. However, the use of KDE gave significantly smaller estimates of home range size for both peripheral (Wilcoxon n=6, $Z = 2.201$, $p = .028$) and core areas (Wilcoxon n=6, $Z = 2.201$, $p = 0.28$) compared to harmonic mean estimators, as detailed in Figure 5.12.

5.2.4 - Discussion

5.2.4.1 - Range use

In this pilot study, range use of *P. auritus* was characterised by small home ranges and high site-fidelity. Small home range size is consistent with previous findings from radio-tracking studies on *P. auritus* by (Fuhrmann and Seitz, 1992; Entwistle et al., 1996; Murphy et al. (in prep.). The radio-tracking in this study was carried out over a mean radio-tracking period of 4.10 hours per night per individual (SD = 1.32; n = 6). Although asymptote was reached for four of the individuals, it is possible that a larger area would have been obtained for each bat if radio-tracking had taken place throughout the entire night. During the three nights that the six bats were radio-tracked, all returned to the same feeding areas on consecutive nights and each remained loyal to a particular area. Given the high consistency of range use it is considered that the identification of core and peripheral foraging areas is meaningful for *P. auritus* during a short term of radio-tracking study.

5.2.4.2 - Effect of transmitters on bat behaviour

A study by (Gaisler et al., 1980 - 1981) found that their survey methods, which included the capture and handling of bats, were negatively influencing the bat population they were studying. However, a study by (Hickey, 1992) compared the foraging success of hoary bats, *Lasiurus cinereus*, with and without radio-transmitters and found that there was no significant difference. Ringed and radio-tracked bats have been re-found year after year (Kurta and Murray, 2002; Neubaum et al., 2005; Greenaway, 2008) and the general hardiness and longevity of these animals may indicate that this is not a problem. During the study, although

fixes from the first night of radio-tracking were omitted from the analysis, all bats returned to the area in which they had been captured and utilised the same foraging areas on subsequent nights which suggests that capture, handling and tagging did not unduly affect their behaviour. Nonetheless, it cannot be ruled out that stress, due to handling and marking, may have altered the individual's behaviour.

5.2.4.3 - Radio-tracking technique

Normally a radio-tracking study requires a triangulation technique, with two or more direction bearings obtained from receivers at known locations (White and Garrott, 1990). Due to a lack of manpower available during the pilot study, the only available methodology was to take single bearings using one receiver at various locations around the animal's foraging range. Even though the minimum time possible was left between taking bearings, so that the bats movement in this time was also there was the potential for some of the estimated locations to be inaccurate due to the movement of the bat in the time between each of the bearings being recorded. Sometimes bats flew rapidly from one location to the next and, therefore, it was considered that the single bearings approach may be inappropriate for this species and triangulation would yield more accurate results.

5.2.4.4 - Home range estimators

The implementation of any home range estimator will have an important effect on the results of estimated home range use in any radio-tracking study. The use of the harmonic mean methodology for the analysis of home range demonstrated that when individuals, such as Bleb 7, foraged along hedgerows, the home range area estimated appeared greatly over-

inflated in size compared to the distribution of the actual estimates of locations. A re-analysis of the estimates of locations, using Kernel Density Estimators (KDE), showed that the resulting areas better matched the distribution of the fixes. The Kernel method produces a nonparametric estimation of a distribution range based on a random sample of point observations. Each kernel is a density, so the resulting distribution range estimate is a true probability density function (Worton, 1989). The selection of a fixed bandwidth (h), however, may have important consequences for range estimates (Gitzen et al., 2006). A large bandwidth can lead to oversmoothing resulting in a large polygon encapsulating all fixes, with no internal definition, whereas a small bandwidth can lead to undersmoothing resulting in individual rings around each fix (Kenward, 2001). Undersmoothing is likely to have occurred in the analysis of KDE, particularly for Bleb 2 (see Figure 5.1) which had a low number of fixes.

5.2.5 - Conclusions

P. auritus used the same feeding areas on consecutive nights and each remained loyal to particular foraging areas. All night radio-tracking from dusk to dawn (as opposed to a fix time schedule) would increase the likelihood of incorporating all feeding localities during the foraging period. Radio-tracking from two positions, with two observers (communicating via two way radios) would allow for simultaneous and more accurate bearings to be collected.

Analysis of home range methodologies revealed that kernel density estimators better matched the distribution of *P. auritus* fixes compared to harmonic mean methodologies. However, the selection of an appropriate bandwidth (h) may have important implications for final range size estimates.

5.3: Ranging behaviour of female *P. auritus*

5.3.1 – Introduction

The pilot study, described in Section 5.2, established the appropriate methodological techniques and subsequent analysis. This was used to inform a detailed radio-tracking study of female *P. auritus* described in this section.

5.3.2 - Methods

5.3.2.1 - Study Area

The study area consisted of seven woods at four sites, three in West Sussex and one in East Sussex (Figure 5.13). All sites were in broadleaved deciduous woodland dominated by oak. NVC analysis identified all the woods as being W10 *Quercus robur, Pteridium aquilinum, Rubus fruticosus* woodlands. This is the most common woodland type on lowland sites in south east England (Hall, 2004). The sites were chosen because previous work had indicated the presence of reproductive *P. auritus* females (Murphy *et al.* in press; unpublished data). One wood was owned and managed by the Sussex Wildlife Trust and six were privately owned. The woods were managed for a variety of purposes including forestry, pheasant shooting and wildlife conservation. All of the woods had good cover in the canopy layer and a well developed understorey of woody shrubs (see Murphy *et al.* in prep for further detailed of woodland structure and *P. auritus* use of woodlands).

5.3.2.2 - Capture and selection of study animals

Between July 2007 and September 2008 a total of 24 female *P. auritus* bats were captured at the study sites and fitted with radio-transmitters. Bats were caught in either mist nests or harp traps, attracted by the use of an acoustic lure (Sussex Autobat: Hill & Greenaway, 2005) that produced synthesised social calls of *P. auritus* (as detailed in Chapter 4). Capture sites were located in the woodland interior, away from edges and rides, and trapping began approximately 60 minutes after sunset. These measures were taken to maximise the chances of catching bats that were foraging rather than ones commuting to or from their roosts. Trapping for bats was suspended in both years from 20th June – 10th July in order to avoid causing distress to heavily pregnant females or separating adult females from their young pups.

5.3.2.3 - Radio-transmitters and tracking methods

Each female was fitted with a Holohil LB-2 (Holohil Systems Ltd.) radio-transmitter. Fur was clipped from the area between the bat's shoulder blades and the transmitter was attached using Skin-Bond ® (Smith & Newphew Inc) surgical adhesive. The tags weighed 0.5 – 0.6g including glue, which was equivalent to 7% of body mass (average across all females; range 5.9% and 7.2%) and was always less than 10%, as recommended by Wilkinson & Bradbury (Wilkinson and Bradbury, 1988). Radio-tracking records indicate that tags usually fall off the animals after 5 - 12 days (unpublished records).

The animals were radio-tracked on foot, by two surveyors, using Sika radio-receivers (Biotrack Ltd) and Yagi 3-element antennas mounted on portable masts with an adjustable height up to 4.2m, to increase the detection of the signal due to the unevenness of the terrain.

The position of the focal bat(s) was communicated by the surveyors using two-way radios (Motorola AU1200). The bat's position was determined by synchronised triangulation from various known locations (determined by GPS Garmin eTrex Legend HCX) around the bat's foraging area. Time, compass bearing, location, weather conditions and notes were recorded on data sheets in the field. These details were recorded approximately every 5 minutes when in contact with the focal animal(s) during the tracking period, with the aim of providing estimates of location that could be plotted onto mapping software and analysed. The surveyors took 15 minute recording breaks approximately every two hours but tried to synchronise this with periods of the focal bat(s) inactivity. In 2007 and 2008 a total of 10 pairs of female *P.auritus* (termed dyads) were radio-tracked synchronously (see Chapter 7 for detailed analysis for range overlap and interaction analysis). Synchronous radio-tracking involved taking a set of bearings from one individual from the dyad followed immediately by a set of bearings from the other member of the dyad.

The effect of autocorrelation (i.e. correlation between the locations of successive 'fixes') on home range estimates has been the subject of debate amongst researchers. Several authors have argued that frequent monitoring of an individual's location via radio-tracking or direct observation severely jeopardises the validity of the independence assumption (Dunn and Gipson, 1977), and that frequent successive observations will tend to be positively correlated which results in a underestimate of the true size of the home range (Swihart and Slade, 1985a; Swihart and Slade, 1985b; Harris et al., 1990).

However, more recently, it has been argued that autocorrelated data is needed to adequately represent the non-random movement that many animals exhibit (Rooney et al., 1998; De Solla et al., 1999; Otis and White, 1999). Using simulated data, de Solla *et al.* (1999)

demonstrated that the accuracy and precision of home range estimates improved at shorter time intervals despite the increase in autocorrelation among the observations. They concluded that the ideal data collection regime would be one where data was collected as frequently as possible, at evenly spaced intervals throughout the study period. Therefore, in this study, the aim of the tracking was to obtain as much information on the bat's use of home range during the short period of radio-tracking (due to constraints on the lifespan of the radio-transmitters used which, in turn, is determined by the body mass of *Plecotus auritus*). Although this meant that data points for radio-tracked individuals were temporally correlated, it was the only practical method available given the short duration of the study. The potential bias of collecting data on unrepresentative nights (nights where low temperatures ($< 8^{o}$ C) and/or excessive rainfall (more than 30 minutes of continuous rain), resulting in extended periods of inactivity) was minimised by excluding fixes near roost locations and during periods of inactivity (i.e. where the bat was 'hanging up').

There are biases to radio-tracking studies that need to be accounted for when analysing species foraging ranges. When two bearings are used to triangulate a tagged animal's position, errors can arise in several ways (Heezen & Tester, 1967; cited in Kenward 2001). Systematic errors can occur from misalignment of the compass, or readings taken when the compass is too close to a large metal object (such as the receiver) (Kenward, 2001). Some bearings may also not triangulate accurately which may be a consequence of the bat moving rapidly from one area to another in the time between each bearing being recorded (Schmutz and White, 1990). In this study this source of error was avoided by two field workers taking simultaneous bearings. Nevertheless, approximately 2% of the overall bearings did not triangulate, which may have been a result of human error.

Data from each night of radio-tracking was added to a cumulative database and for each individual this was used to estimate the bat's ranging area (see below). Individual females were radio-tracked for as many nights as was required for the cumulative ranging areas to reach asymptote, as recommended by Kenward (2001) and Laver and Kenward (2008).

5.3.2.4 - Home range analysis

Pairs of compass bearings, and the locations they were taken from, were used to estimate the bat's position (fix) by triangulation with the software package LOAS (version 2.12, Ecological Software Solutions, 1998 – 2003). The fixes obtained from LOAS were imported into Ranges 7 (version 1.8, Anatrack Ltd, 2006) and ArcGIS 9 (version 9.2, ESRI 2006) which were used to produce visual representations of the estimated ranging areas.

Two range estimators were used to calculate total range estimates for each individual: 100% MCP and 95% KDE (see Section 5.2). Kernel analysis was used to produce a probability density surface over fixes (Silverman, 1986). The influence of points is estimated by a bivariate normal estimator and the distance of which a point has an influence is controlled by the bandwidth (h). However, the selection of bandwidth can have a substantial effect on the size of home range estimates (Gitzen et al., 2006). The reference bandwidth (href), which is a function of the variance in the fix co-ordinates, tends to oversmooth and hence overestimate range size if an animal has more than one area of high activity (Worton, 1989). To overcome this problem, Worton (1989) suggested the use of either least-squares cross validation (LSCV) to select an appropriate multiplier of href or, alternatively, allowing the value of h to vary throughout the range (known as adaptive kernel analysis). Therefore, individual home ranges were estimated as adaptive kernels with the smoothing factor *h* estimated from the

data and optimised via least square cross validation, LSCV (h_{cv}; Worton, 1989). The females' home ranges were calculated with a mean h_{cv} to keep home ranges comparable among individuals (Kenward, 2001). The data sets for each bat used for home range analysis contained more than 30 fixes (mean number of fixes = 161.04, SD = 30.57, n = 24) as recommended by (Seaman et al., 1999). (Silverman, 1986) reported a bias in LSCV estimators of clumped data points. However, the exclusion of duplicate fixes at roost and 'hanging up' (i.e. night roosting locations) sites eliminates the risk of overestimation of habitat use around roosts due to, for example, swarming behaviour.

5.3.2.5 - Core areas

Core areas can be a useful concept when describing patterns of behaviour or identifying particularly resources (Harris et al., 1990; Powell, 2000). All bats showed clumping in their use of space (see Figure 5.14) with maxima occurring between the 35% and 65% isopleths (Table 5.3). The 50% isopleth (median value) was adopted as an indicator of core area use. The use of 50% KDE for *P. auritus* core use in this study, would also make it comparable with other radio-tracking studies on woodland bats, for example, Barbastelle *Barbastella barbastellus* (Hillen et al., 2009), (Kerth and Melber, 2009) Bechstein's *Myotis bechsteinii* (Dietz and Pir, 2009; Kerth and Melber, 2009) and lesser horseshoe *Rhinolophus hipposideros* (Bontadina et al., 2002). The 50% isopleth was, therefore, adopted as an indicator of core areas.

5.3.2.6 - Statistical analysis

The emergence time of radio-tagged females in relation to sunset and civil twilight was examined using regression analysis. For the analysis of radio-tracking data, separate analysis of variance (ANOVASs) was carried out for the mean MCP, mean peripheral foraging area and mean core foraging area. Reproductive status (pregnant, lactating, post-lactating) was entered as a factor in order to test for any association between reproductive condition and range size. Data was transformed so that the residuals were normally distributed whenever possible.

5.3.3 - Results

Radio transmitters were attached to a total of 24 female bats and data was obtained from all 24 radio-tracked individuals. Details of female *P. auritus* radio-tracked over the two years are shown in the Appendix Table A.6.1. The 24 bats were radio-tracked over 56 nights from emergence to re-entry, resulting in a total of 3865 fixes that were used for analysis. On 36 of these nights, a total of 20 of the 24 individuals were radio-tracked simultaneously in pairs (see Chapter 7).

5.3.3.1 - Nocturnal activity of radio-tagged females

Broadly speaking, the nocturnal behaviour of *P. auritus* could be defined as one of three activities.

i) Foraging: Bats spent time flying in discrete locations (termed foraging areas) and during this activity it was assumed that the bat was seeking or consuming prey. Within these areas, bats were occasionally observed flying slowly in and out of trees with a dipping flight path, as described by (Entwistle et al., 1996).

ii) Resting: Females were assumed to be inactive or 'hanging up' when the signal was steady without amplitude modulations which typifies a moving animal (Burger et al., 1991; Krull et al., 1991; Kenward, 2001).

iii) Commuting: This typically involved fast directional flight to and from the roost site.

The 24 bats radio-tracked in the study utilised a combination of tree and house roosts (see Murphy *et al.* in prep for detailed analysis of differential characteristics between roosting in tree and house roosts). The median emergence time of radio-tagged bats occurred 38 minutes after sunset (mean = 42.1 min, SD = 27.5, n = 81) and 6 minutes before the end of civil twilight (mean = 7.4 min, SD = 26.65, n = 81). The times of emergence for each female, on each night, in comparison to sunset, are shown in Figure 5.15 (a) and in comparison to civil twilight are shown in Figure 5.15 (b). The time of emergence was highly correlated with both the time of sunset (Spearman rank correlation, $r_s = 0.878$, n = 81, $p < 0.01$) and with the time of civil twilight (Spearman rank correlation $r_s = 0.875$, n = 81, $p < 0.01$). However, two individuals, one adult and one juvenile (Bleb 25 and 26) simultaneously radio-tracked, utilised a tree roost together within their foraging range, and emerged later than other tagged females (up to 2.5 hours after sunset) during the radio-tracking period (which may have been as a consequence of adverse weather conditions during that period).

The median time for final re-entry to roost was 36 minutes before sunrise (mean = 38 min, SD = 14.12, n = 52) and 1 minute after the beginning of civil twilight (mean = 1 min, SD 10.62, n = 52). The times of final re-entry to roost for each female, on each night, in comparison to sunrise, are shown in Figure 5.16 (a) and in comparison to the civil twilight are shown in Figure 5.16 (b). The time of returning to the roost was also highly correlated with both the time of sunrise (Spearman rank correlation $r_s = 0.940$, n = 52, p <0.01) and with the time of civil twilight (Spearman rank correlation $r_s = 0.932$, n = 52, p <0.01). The times of emergence varied between roost sites but there was so significant difference between mean emergence times of females roosting in trees compared to females roosting in buildings (Kruskal-Wallis ANOVA H = .142, n = 24, p < 0.706), and the mean time of emergence for each roosting location was not significantly correlated with the distance to the closest woodland (Spearman rank correlation $r_s = 0.038$, n = 24, p < 0.858).

The nocturnal activity pattern of females was characterised by bouts of flight combined with short periods of inactivity, as described by Entwistle et al. (1996). The periods of inactivity, ranging from one minute to 85 minutes (median = 16 min), were predominately spent in trees (seventy two occasions), including trees used as day roosts and, less often (four occasions), in buildings used as day roosts. Each of the buildings used was situated within or at the edge of broadleaved woodland. Both lactating and non-lactating females re-entered the maternity roost during the night, but lactating females (n = 6) re-entered more frequently compared to pregnant, non-reproductive and juvenile females (n = 18) (Kruskal-Wallis ANOVA H = 13.66, n = 24, p < 0.001). Excluding the lactating females (as periods of inactivity could not be separated from time feeding offspring), the mean hours of rainfall recorded during the radio-tracking period was significantly correlated with mean time spent inactive (Spearman rank

correlation r_s =0.860, n = 18, p < 0.01) as shown in Figure 5.17 (a) and negatively correlated (although weaker) with mean minimum temperature recorded during the radio-tracking period (Spearman rank correlation r_s = -0.683, n = 18, p < 0.01) as shown in Figure 5.17 (b). A partial correlation between inactivity and rainfall (controlling for temperature) did not find a significant correlation (r = -0.389 n = 18 p = 0.123). However, a partial correlation between inactivity and temperature (controlling for rainfall) did find a significant correlation (r = 0.693 n = 18 p = 0.002), which suggests that temperature may be a more important determinant of inactivity compared to rainfall.

5.3.3.2 - Asymptotes of range size

The point at which the asymptote in the cumulative size of the bat's foraging range was reached was used to indicate if sufficient data had been gathered from each bat to provide an estimate of the extent of home range use. Asymptotes of home-range size were achieved for 22 of the 24 females radio-tracked. Blebs 31 and 32, radio-tracked in September 2008, did not reach the asymptote, but it was decided to terminate radio-tracking as adverse weather conditions during that period may have resulted in the tags failing. Range size plot of all females radio-tracked in 2007 are shown in Figure 5.18 and reveals that range size begins to level off at about 60 fixes and reaches a more stable estimate after ca. 115 fixes.

5.3.3.3 - Foraging characteristics

All individuals foraged within broadleaved woodland and some individuals also utilised mature hedgerows. For all females the majority of foraging fixes were recorded in woodland (including copses) (see Murphy *et al.* in prep for detailed analysis relating to habitat use).

Hedgerows were also utilised by females and there was evidence of seasonal difference in use (see Murphy *et al.* in prep). Most fixes in the other habitat types were associated with trees. For example, gardens all abutted woodland and had trees in them, and bats recorded over water were generally within a few metres of the tree-lined bank of a stream or the edge of a woodland pond or lake. Figure 5.19 shows the spatial distributions for four females, which were radio-tracked at Plashett Wood in 2007 (overlaid on an OS map of the area). The 95% KDE and 50 % KDE demonstrates that females predominately focus their foraging in broadleaved woodland.

5.3.3.4 - Home range size

Minimum Convex Polygon (100%) home ranges of individual females varied between 2.7 ha and 19.42 ha (mean = 7.42 ha, n = 24, SD = 3.47; Table 5.4). Kernel density estimates of home ranges of individual females varied between 1.64 ha and 10.16 ha (mean = 4.54ha, n = 24, SD = 1.99; Table 5.4). Reproductive status (pregnant, lactating, post-lactating) had no significant effect on range size regardless of which type of range estimator was used (MCP: $F=0.294$, d.f. = 23, $p = 0.651$; 95% KDE: $F = .165$, d.f. = 23, $p = 0.514$). Figure 5.20 shows the spatial distribution of the 100% MCP and Figure 5.21 shows the spatial distribution of the 95% KDE for eight female radio-tracked in Plashett Wood in 2007. There was overlap between the ranges of different individuals radio-tracked within the same year (including those radio-tracked simultaneously, analysed separately in Chapter 7).

5.3.3.5 - Core area use

Core areas, as defined by the 50% KDE, were areas individual *P. auritus* females used on successive nights and these were characterised by between one and seven locations within their home range (median = 2). These core areas of individual females varied between 0.69 ha and 5.39 ha (mean = 2.38 ha, n = 24, SD = 1.08). In general, females tended to return to these areas to forage on a regular basis each night. Reproductive status (pregnant, lactating, post-lactating) had no significant effect on core area (50% KDE: F = .172, d.f. = 23, p = 0.492). Figure 5.22 shows the spatial distribution of the 50% KDE for each female radio-tracked in Plashett Wood in 2007. There was, also, overlap in core area use between different individuals radio-tracked within the same year (including those radio-tracked simultaneously, analysed separately in Chapter 7). The mean size of all home range estimators (100%MCP, 95%KDE and 50% KDE) for all females radio-tracked in 2007 and 2008 is shown in Figure 5.23.

5.3.4 - Discussion

5.3.4.1 - Nocturnal activity of radio-tagged females

The median emergence time of *P. auritus* in this study was 38 minutes after sunset at 51^0N, similar to the median emergence time of *P. auritus* at the same latitude found by (Battersby, 1999) (36.4 minutes after sunset) and by (Howard, 1995) (40 minutes after sunset) and followed the pattern of other studies whereby median bat emergence was later with increasing latitude: 46^0N, 15 minutes after sunset for a study in France, (Barataud, 1990);

50^0N, 26 minutes after sunset for a study in Germany (Fuhrmann and Seitz, 1992); and at 57^0N, 55 minutes after sunset for a study in Scotland (Entwistle et al., 1996).

The emergence time for bats generally tends to be highly synchronised with the time of sunset (Swift, 1980; Catto et al., 1995; Duverge et al., 2000; Petrzelkova et al., 2006; Reichard et al., 2009) and is likely to be related to the use of light levels as a cue for emergence (Erkert, 1978; Erkert, 1982; McAney and Fairley, 1988; Isaac and Marimuthu, 1993). The evening emergence time appears to be a function of dietary specialisations and foraging strategy and is probably, also, affected by the ability to avoid predation (Jones and Rydell, 1994; Speakman et al., 2000; Reichard et al., 2009).

The median time of roost return of females in this study was 36 minutes before sunrise (1 minute after the beginning of civil twilight). This contrasts with studies carried out in Germany (50^0N) by (Fuhrmann and Seitz, 1992) where *P. auritus* (n = 6) on average returned 186 minutes before sunrise, and also in Scotland (57^0N) by Entwistle et al., (1996) where *P. auritus* (n = 16) returned on average 57 minutes before sunrise. In this study, all the roosts were located either within trees in the woodland or buildings in or at the edge of woodland, connected directly to the woodland by hedgerows and copses. Bats roosting in woodland are less vulnerable to predators and may take advantage of this by prolonging foraging (Russo et al., 2007).

The females roosting outside of the woodland in which they were caught predominately used hedgerows and tree lines for commuting to and from their roosts and foraging sites. The use

of linear features for commuting by bats has been demonstrated for a number of different species including *P. auritus* (Entwistle et al., 1996), *Pipistrellus pipistrellus* and *P. pygmaeus* (Downs and Racey, 2006) and *Rhinolophus hipposideros* (Stone et al., 2009). The use of covered flyways by *P. auritus* may help in avoiding avian predators such as tawny and barn owl (Speakman, 1991).

In Southern England, females flew throughout the night with no substantial period of night roosting, similar to *P. auritus* in Scotland (Entwistle et al., 1996) and other gleaning bats such as *Myotis bechsteinii* (Fitzsimons et al., 2002; Dietz and Pir, 2009), *Myotis evotis* (Chruszcz and Barclay, 2003) and *Myotis myotis* (Rudolph et al., 2009). In contrast to the study carried out in Scotland (Entwistle et al., 1996), both lactating and non-breeding females returned to the roost during the night. However, the fact that in this study female *P. auritus* roosted in trees, often within their foraging range, indicates that the non-lactating females that returned to their roost may have been resting or avoiding adverse weather conditions. Inactivity throughout the night was significantly correlated with temperature and rainfall but when these effects were controlled for, inactivity was significantly was correlated with temperature only. This is in contrast to foraging behaviour in Scotland where inactivity was significantly correlated with rainfall but not temperature (Entwistle et al., 1996). However, the study in Scotland had a small sample size for individuals night roosting on cold nights (n = 7) and it is likely that both temperature and rainfall will have effects on bat activity in different environments and/or habitats (Parsons et al., 2003).

5.3.4.2 - Feeding site fidelity

The knowledge of patches of habitat where high prey availability can be predicted may be a key benefit of remaining loyal to a foraging range, as demonstrated by other animals such as birds (Greenwood and Harvey, 1976; Irons, 1998; Raymond et al., 2010), whales (Valenzuela et al., 2009) and sea lions (Chilvers, 2008). During the time that the 24 female *P. auritus* were radio-tracked, each remained loyal to a particular foraging area. High consistency from night to night in the use of foraging areas has also been shown for bats such as *Macrophyllum macrophyllum* (Meyer et al., 2005) and *Barbastella barbastellus* (Hillen et al., 2009). Other gleaning bats, such as *Myotis blythii* and *M. myotis* (Arlettaz, 1999), *M. emarginatus* (Krull et al., 1991) and *Plecotus austriacus* (Fluckiger and Beck, 1995) have also been shown to use similar sites on successive nights. Studies on *Myotis bechsteinii* (Kerth et al., 2001) and *Myotis myotis* (Rudolph et al., 2009) and *Barbastella barbastellus* (Hillen et al., 2009) have shown that foraging areas for some individuals remained constant over consecutive years.

This study was only able to ascertain loyalty over a period of a few days and it may be that range-use changes over longer periods, expanding or contracting or even shifting foraging areas completely at certain times of the year. There was evidence of seasonal changes in foraging habitat for *P. auritus* in a study by Murphy et al. (in prep), wherein data from 38 females found that hedgerows were the most frequently used habitats after woodland, although their use varied seasonally, with little use before mid July but increasing significantly in August and September. Seasonal changes in foraging behaviour have also been demonstrated for other bat species such as *Nyctalus leisleri* (Shiel et al., 1999) *Nyctalus noctula* (Mackie and Racey, 2007) and *Rhinolophus euryale* (Goiti et al., 2008).

5.3.4.3 - Home range use

The radio-tracking of bats has limitations, such as the short lifespan of the tags, in that the data does not necessarily reveal a full "home range". However, for the purposes of this discussion the focus will be on home range-use at the time of year when the females were radio-tracked. Further limitations to the accuracy of range representation arise due to the accuracy of the bearings, as this is dependent upon the proximity of the receiver to the transmitter which can affect the accuracy of the calculated fixes. Finally, it cannot be ruled out that, as a consequence of tagging, the bat is distressed. However, the females that were caught and tagged in their foraging area remained foraging in that area on subsequent nights, which would suggest that their behaviour was not unduly affected.

There have been no published studies on estimates of home range size for *P. auritus* to date (although see Murphy et al. in prep for details of 38 females which includes data from the 24 females included in this study). Previous radio-tracking studies on *P. auritus* having focused on distance travelled from roosts and habitat use analysis (Fuhrmann and Seitz, 1992; Entwistle et al., 1996). The mean estimated home range for *P. auritus* (100% MCP 7.4ha) is smaller than the mean home range estimates for other UK bat species such, as *Barbastella barbastellus* in Sussex, (Ebernoe Common Colony mean 100% MCP 779ha; (Greenaway, 2008); *Pipistrellus pipistellus* 100% MCP 168ha (Nicholls and Racey, 2006); 100% MCP 157ha (Davidson-Watts and Jones, 2006) *Pipistrellus pygmaeus* 100% MCP 30ha (Nicholls and Racey, 2006); 100% MCP 146ha (Davidson-Watts and Jones, 2006); *Nyctalus noctula* MCP 100% 820ha (Mackie and Racey, 2007), but is comparable with the range size of sympatric woodland gleaning species, such as *Myotis bechsteinii* 95% MCP 1.21ha

(Fitzsimons et al., 2002) and 100% MCP (including commuting routes and roosting localities) 11.2ha (Schofield and Morris, 1999).

5.3.4.4 - Core foraging areas

In this study, females did not utilise all of their home range with equal intensity and returned to specific areas, termed core foraging areas, within their home range more frequently than others. Presumably, these core feeding areas were associated with increased feeding success. A study by Thompson et al., (2007) on the core feeding areas within the home range of East African chimpanzees *Pan troglodytes schweinfurthii* found that the quality of core areas is associated with variance in reproductive success among female East African chimpanzees, whereas females in neighbourhoods containing more preferred food had elevated ovarian hormone production, shorter birth intervals and higher infant survivorship. Vegetation analysis on the composition of *P. auritus* foraging areas (Murphy *et al* in prep.) showed that there was significantly more understorey in terms of cover and, also, species diversity in core foraging areas compared to peripheral foraging areas. Core foraging areas were often characterised by understorey species, such as *Crataegus monogyna* and *Alnus glutinosa*, that support a much greater diversity of insects than species such as *Carpinus betula* (Kennedy and Southwood, 1984) or the non-native *Rhododendron ponticum* (Cross, 1975), which were often the characteristic species present in peripheral areas. As female *P. auritus* rarely travel further than 2km from the colony roost (Entwistle et al., 1996), regular access to key core foraging areas within this distance may be necessary to successfully raise young.

5.4 – Summary

- Individual females clearly had specific ranging areas that they returned to on consecutive nights, although the extent to which these are fixed over longer periods is not known.
- These ranges were small in size in comparison to the size of published home range size of other bat species. Within the home ranges of females were core areas, which females returned to more frequently compared to other areas within their range.

If female responses to synthesised social calls represent defence of resources, then it would be expected that there are differences in response in relation to where the stimulus is played within their home range.

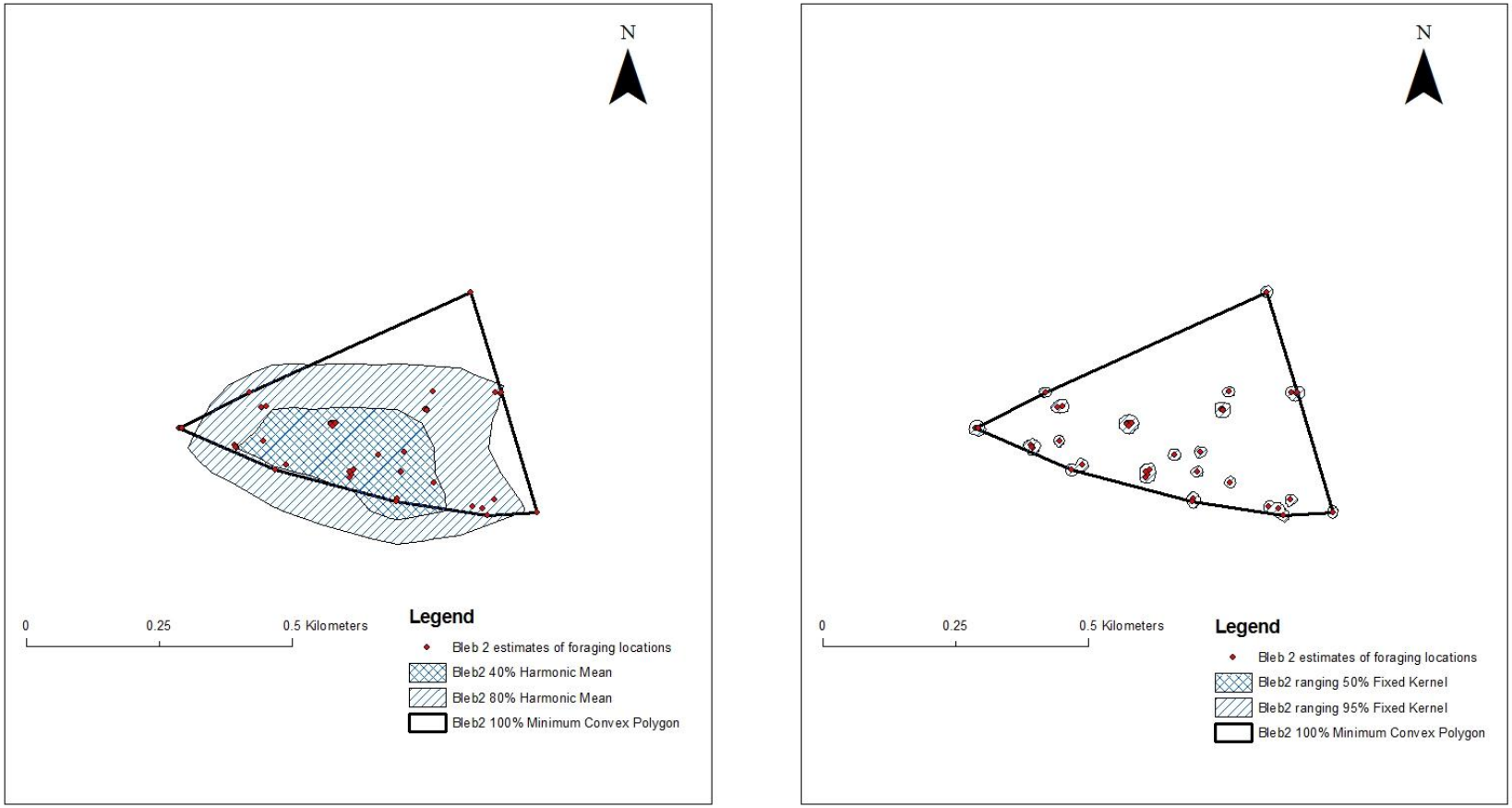


Figure 5.1: Comparative analysis of home range use for bleb 2 using harmonic mean methodology (left) and Fixed Kernel Estimates (right).

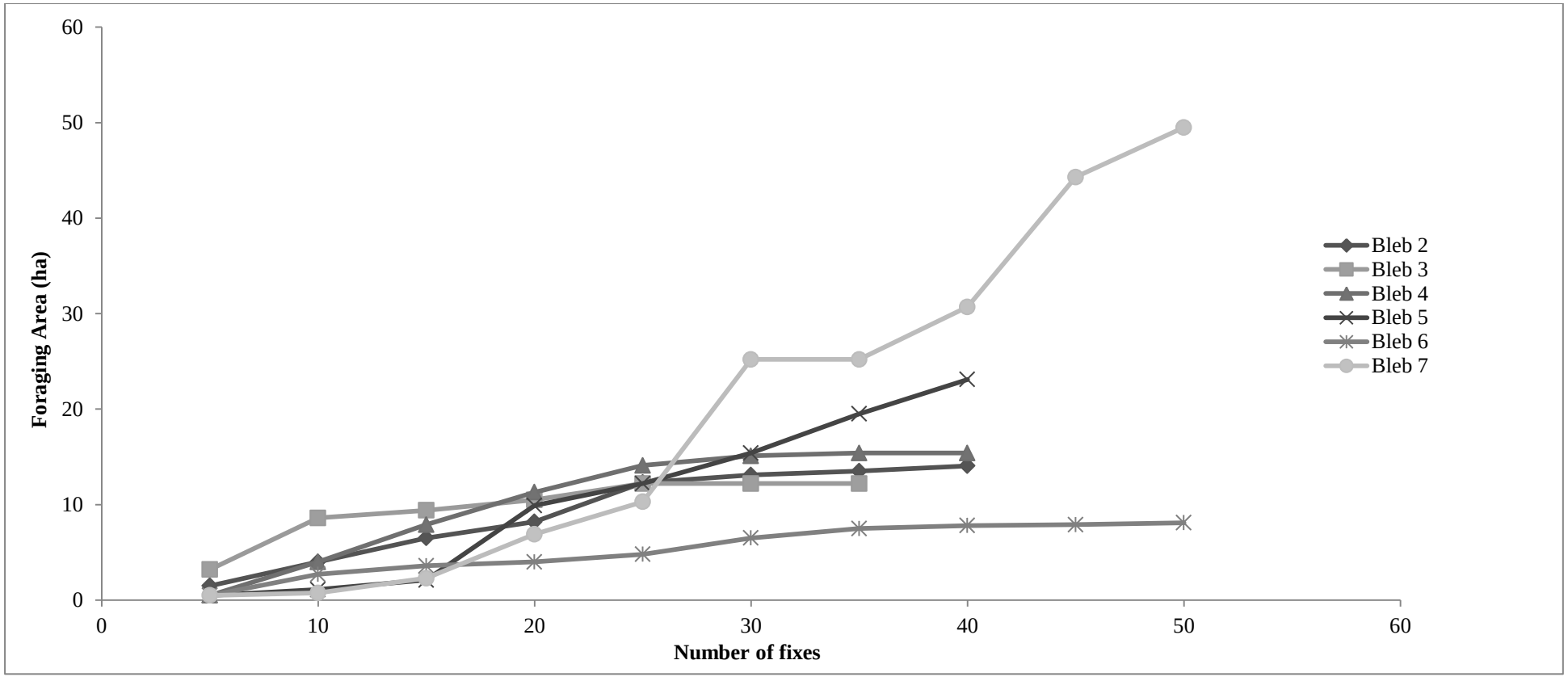


Figure 5.3: Cumulative home range size against number of successive locations for all *P.auritus* radio-tracked in July – Sept 2006. Estimates of range size are based on 100% minimum convex polygons.

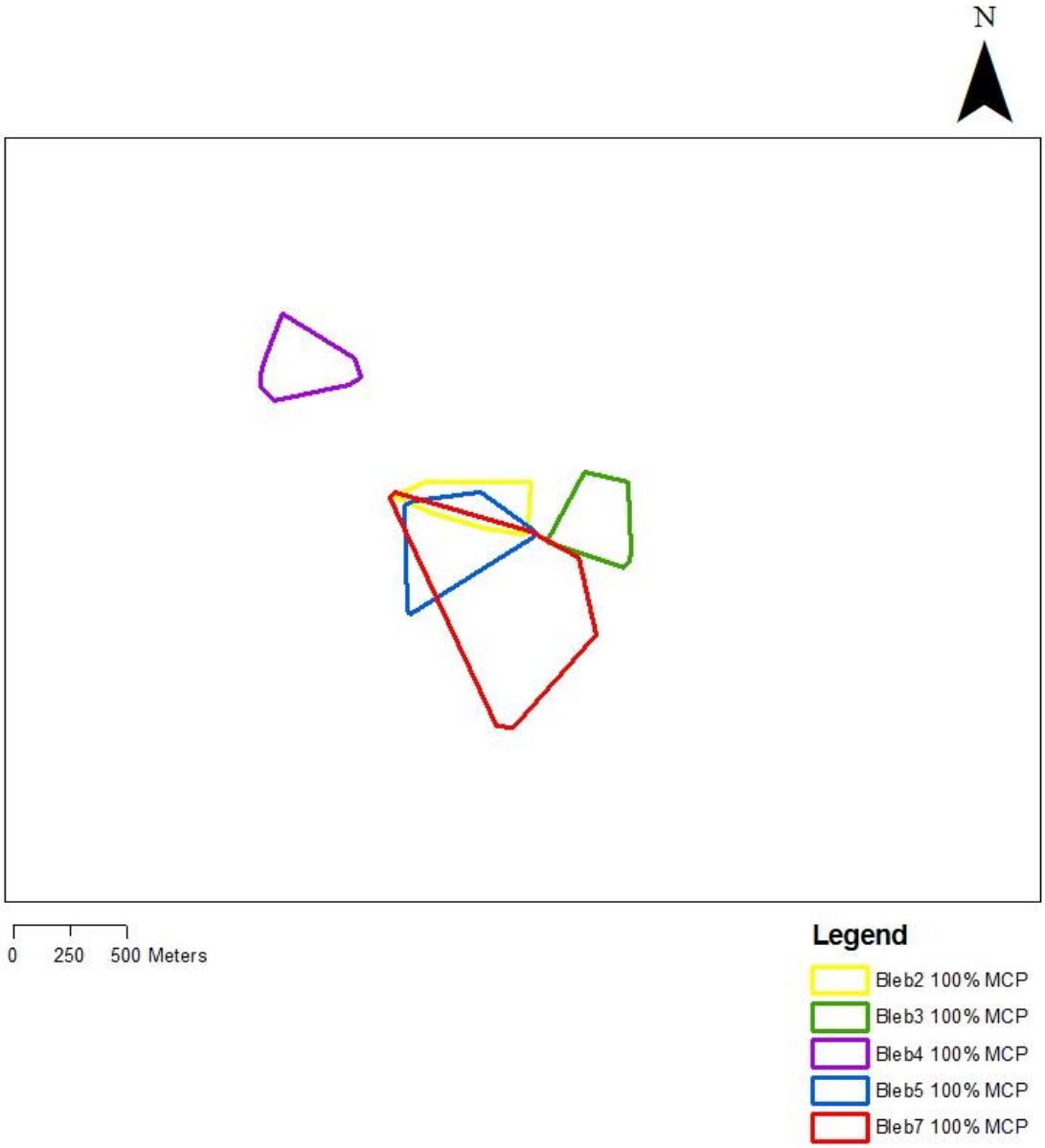


Figure 5.4: 100 % Minimum Convex Polygons home range representation of five *P. auritus* radio-tracked at Plashett Wood in 2006.

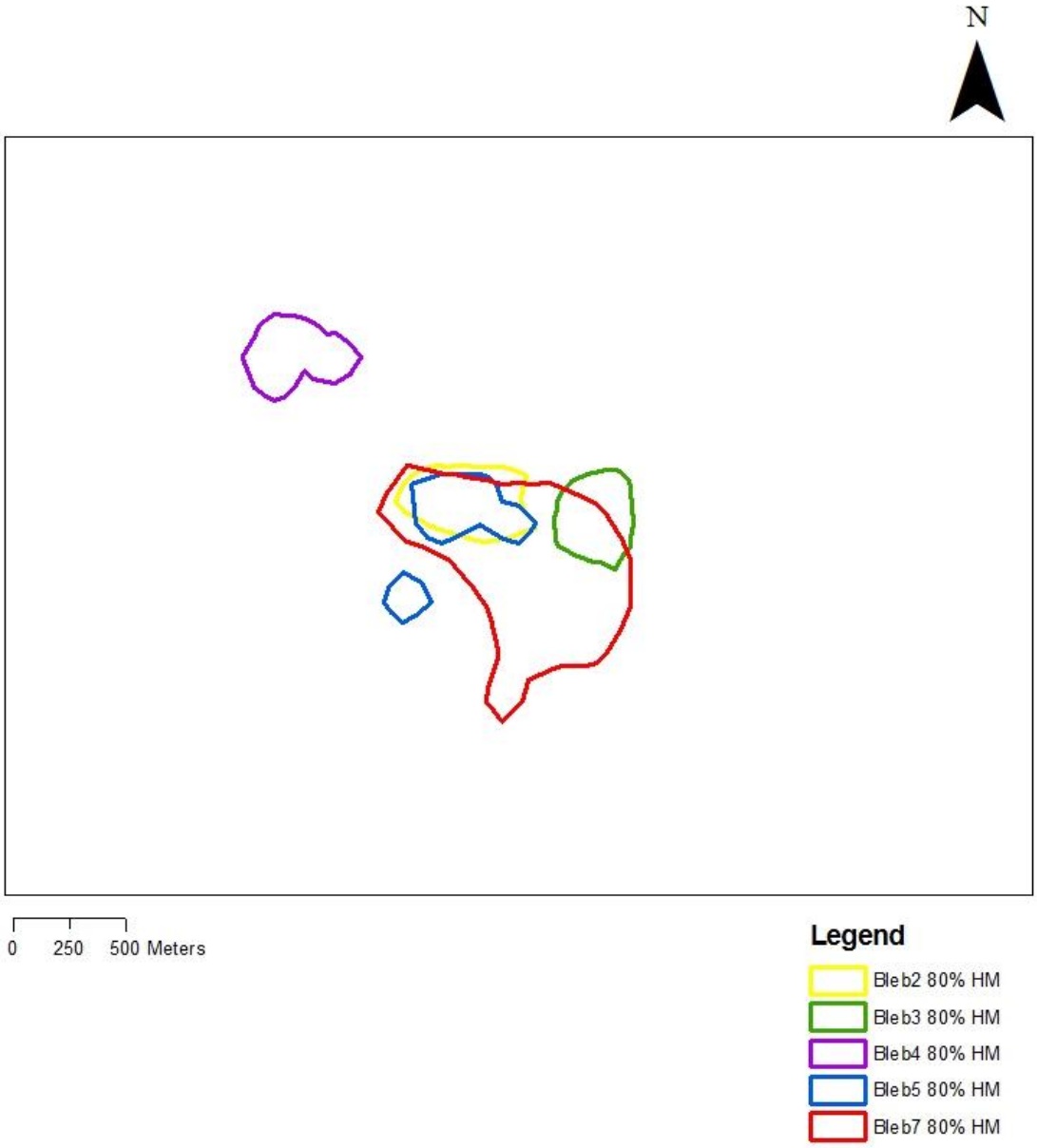


Figure 5.5: 80% Harmonic Mean home range representation of five *P. auritus* radio-tracked at Plashett Wood in 2006.

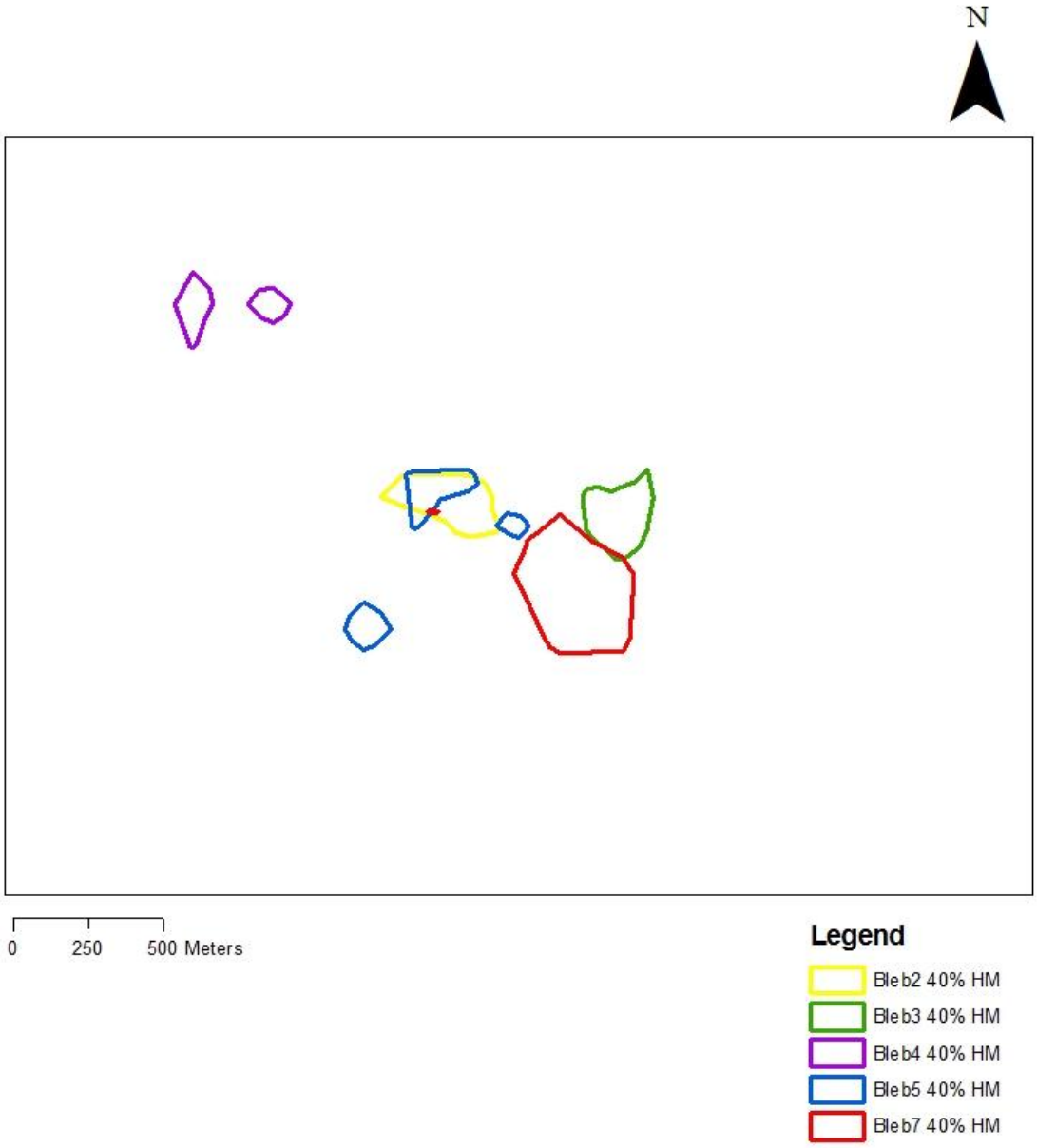


Figure 5.6: 40% Harmonic Mean home range representation of five *P. auritus* radio-tracked at Plashett Wood in 2006.

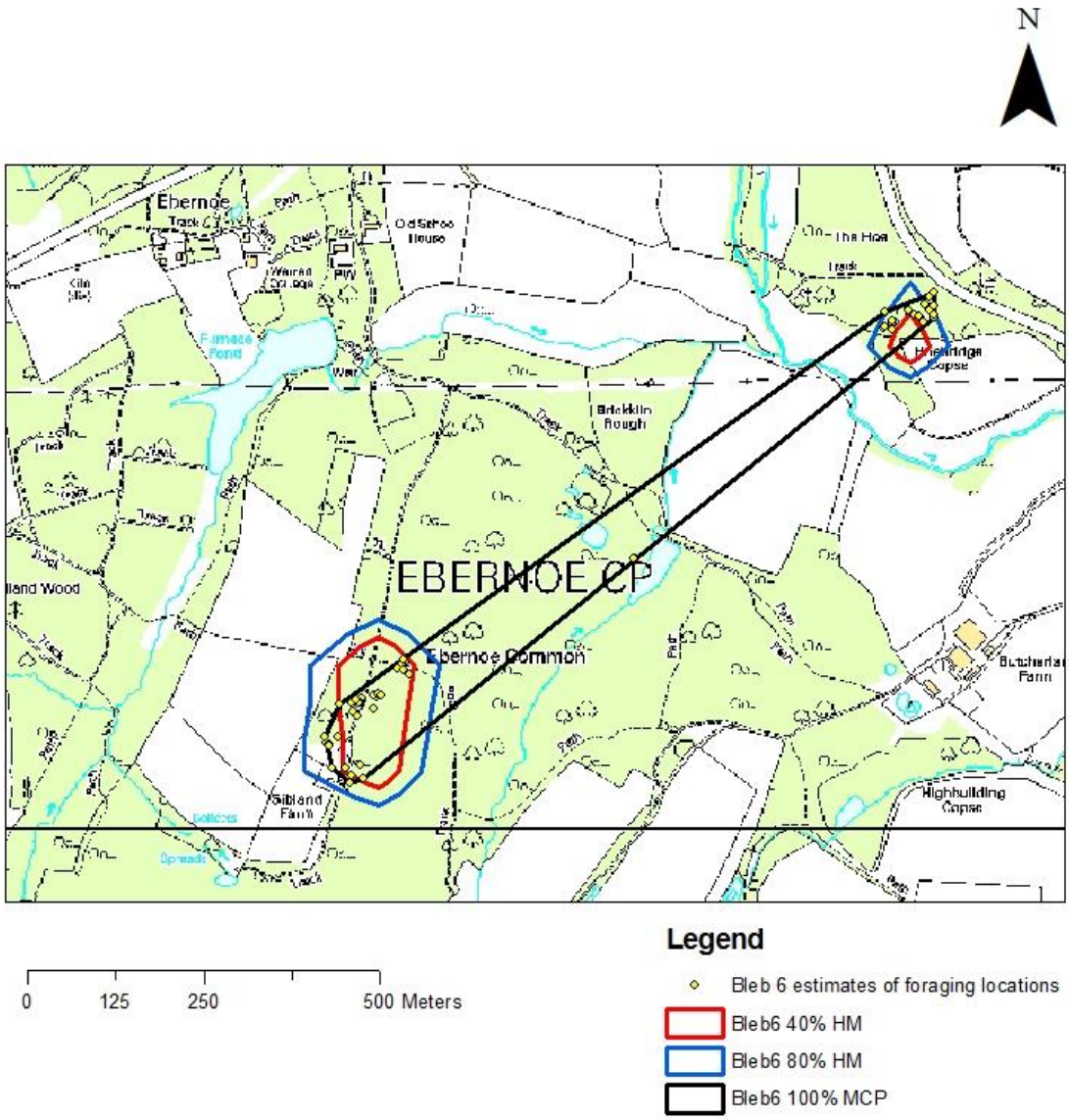


Figure 5.7: Bleb 6 radio-tracked at Ebernoe Common 2006. This female had two clear foraging locations, one in Ebernoe Common adjacent to her roost and a secondary foraging area in The Hoe approximately 1 km from her primary foraging area. The locations for many of her fixes were outside the 40%HM in her secondary foraging area, which illustrated that the home range analysis methodology may not be suitable.

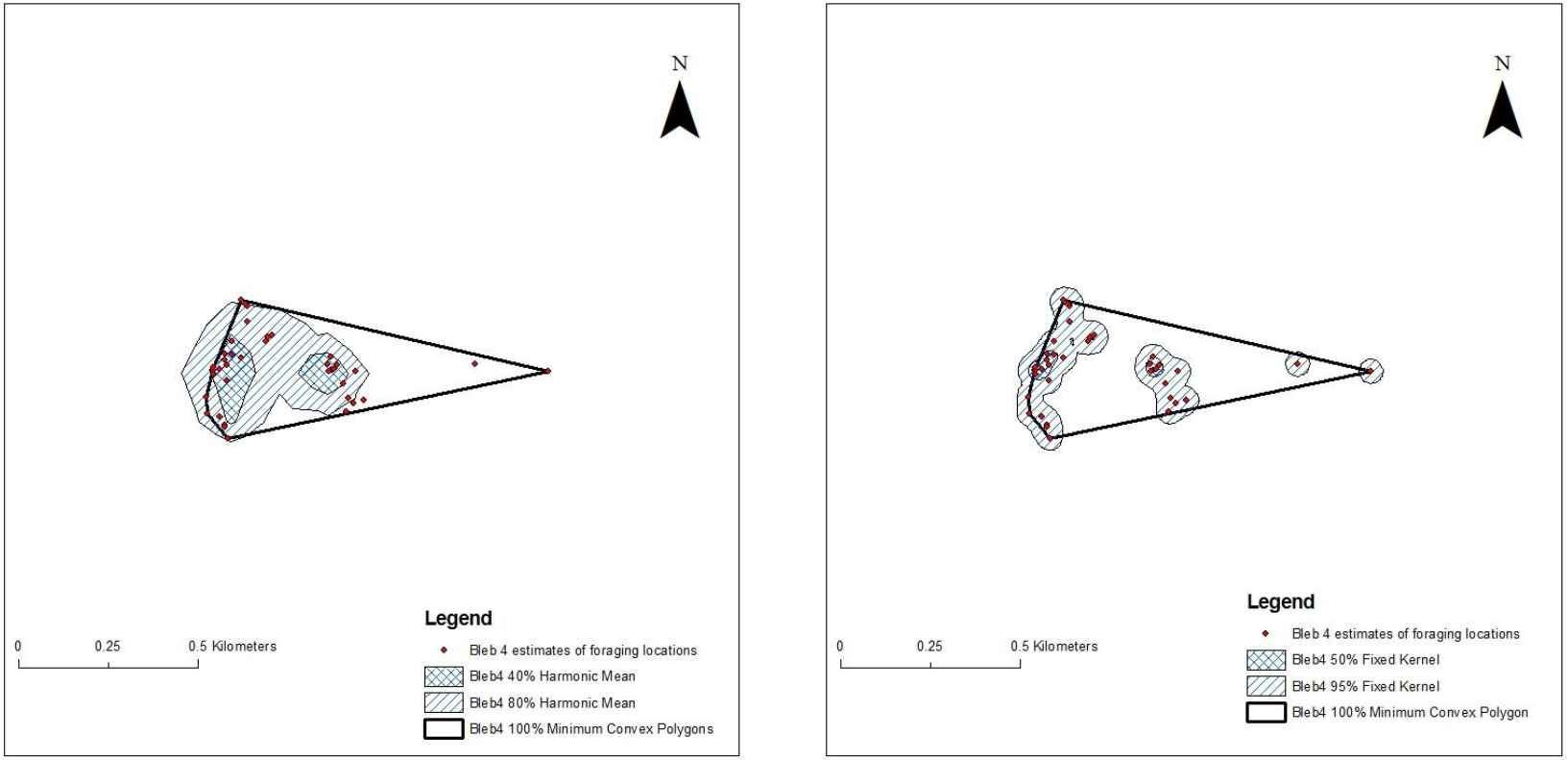


Figure 5.9: Comparative analysis of home range used for Bleb 4 using harmonic mean methodology (left) and fixed kernel estimated (right). The home range estimation of the 80% harmonic mean includes a major trunk road and an arable field situated between the two 40% harmonic mean core areas. These were habitats not frequented by bleb 4 and this is much more clearly represented by the fixed kernel analysis.

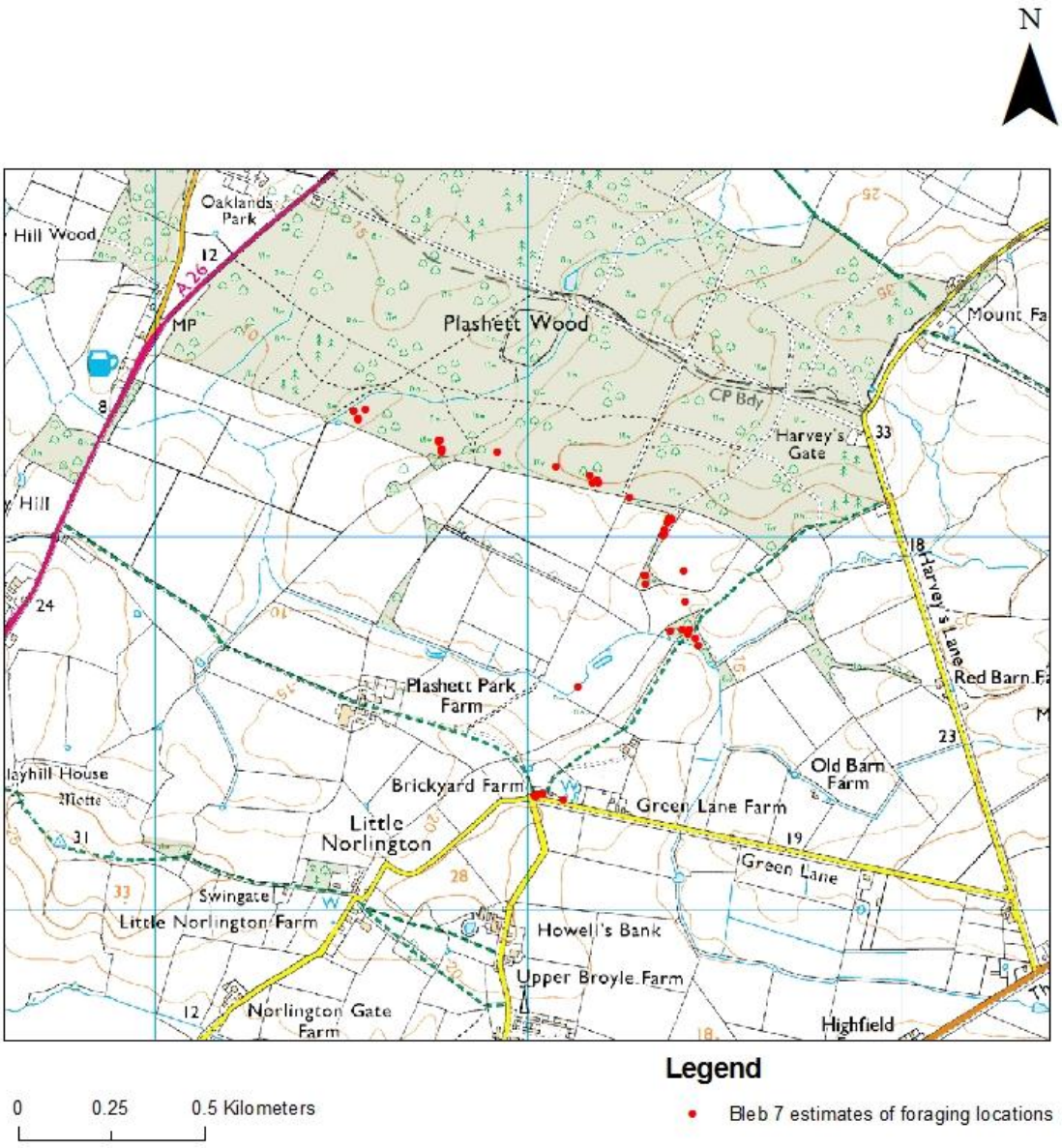


Figure 5.10: Estimates of foraging fixes for Bleb 7. The distribution of the fixes closely matches hedgerows, copses and woodland edge.

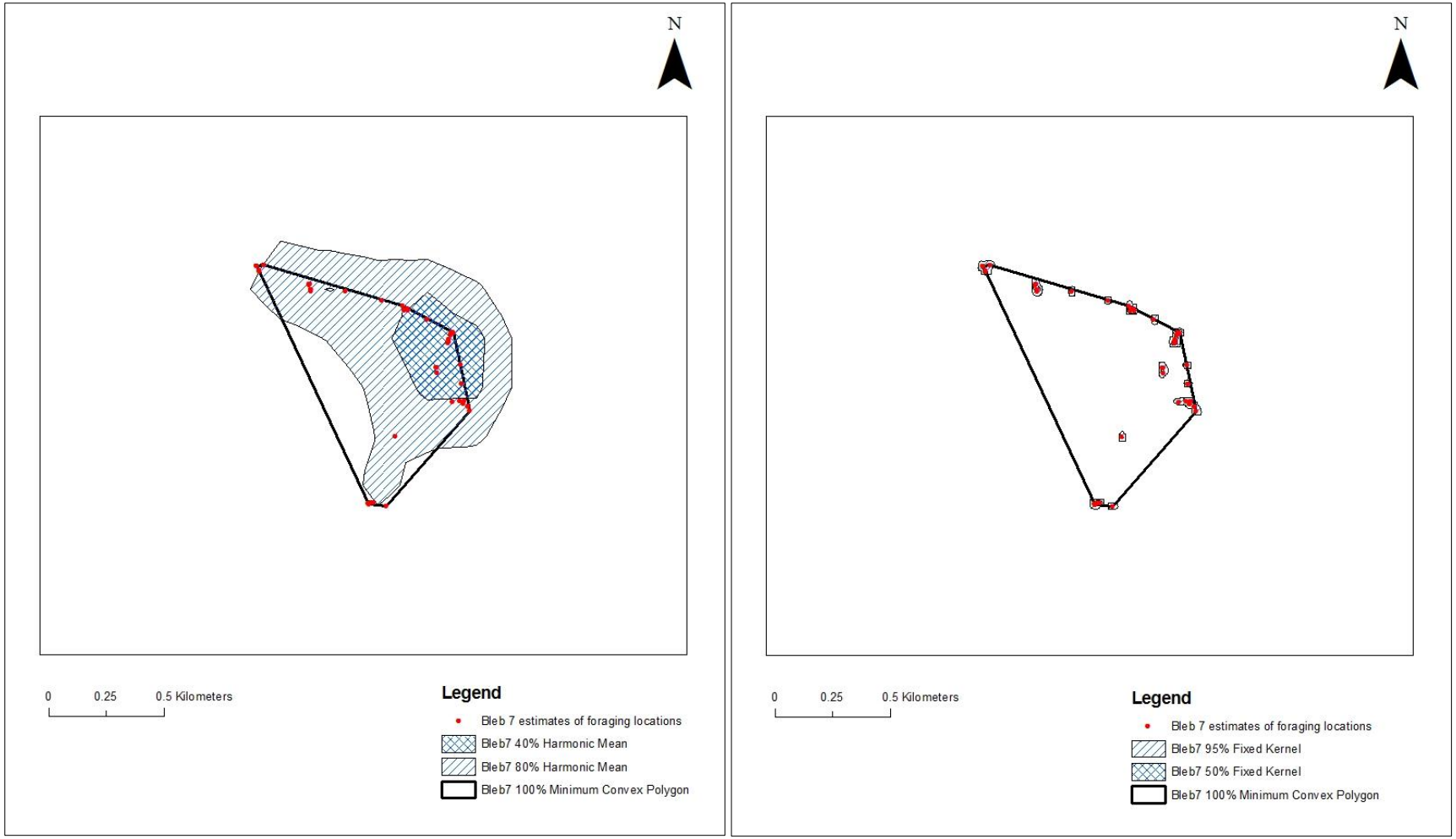


Figure 5.11: Comparative analysis of home range use for Bleb 7 using harmonic mean methodology (left) and fixed kernel estimates (right).

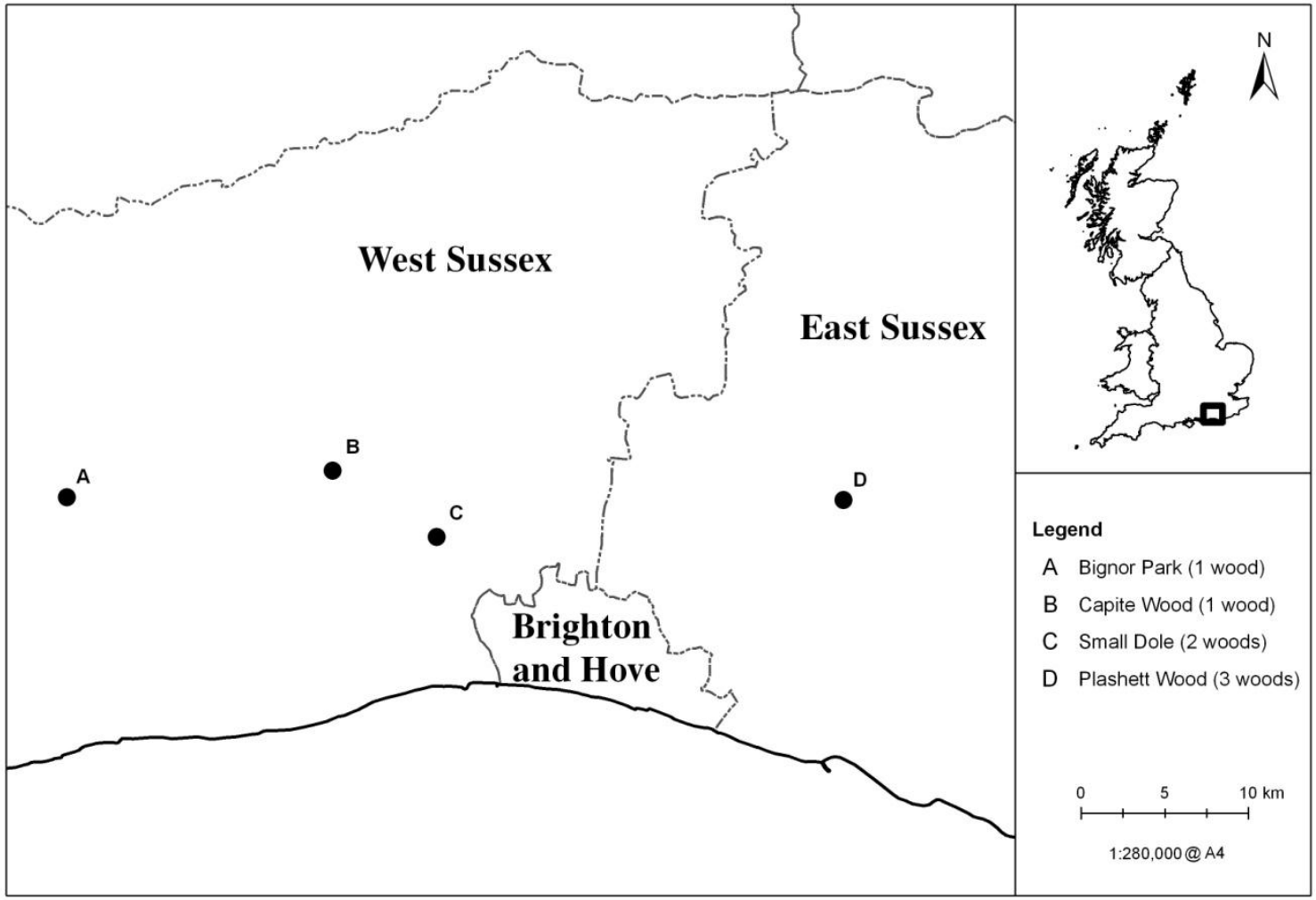
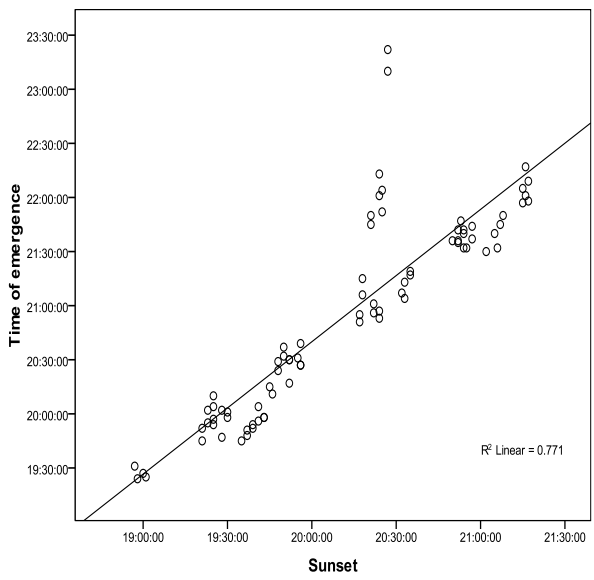


Figure 5.13: Locations of four sites (7 woodlands) where the radio-tracking study of *P. auritus* was carried out in 2007 and 2008.



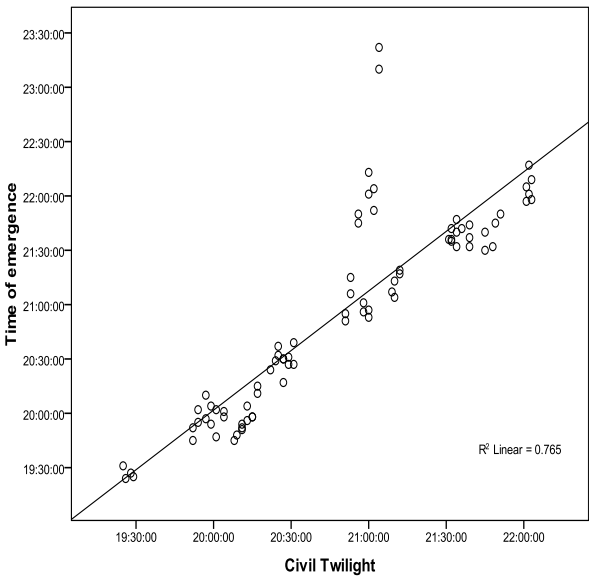
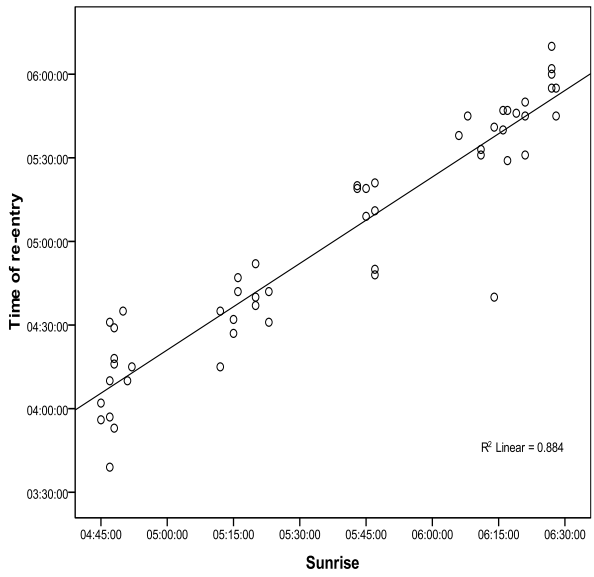


Figure 5.15a (left): Emergence time of radio-tagged female *P. auritus* was significantly correlated with the time of sunset.

Figure 5.15b (right): Emergence time of radio-tagged female *P. auritus* was also significantly correlated with the time of civil twilight.



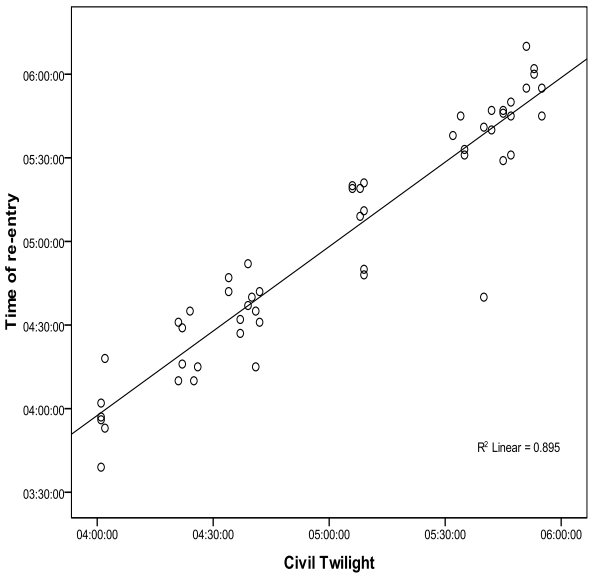


Figure 5.16a (left): Final roost re-entry time of radio-tagged female *P. auritus* was significantly correlated with the time of sunrise.

Figure 5.16b (right): Final roost re-entry time of radio-tagged female *P. auritus* was also significantly correlated with the time of civil twilight.

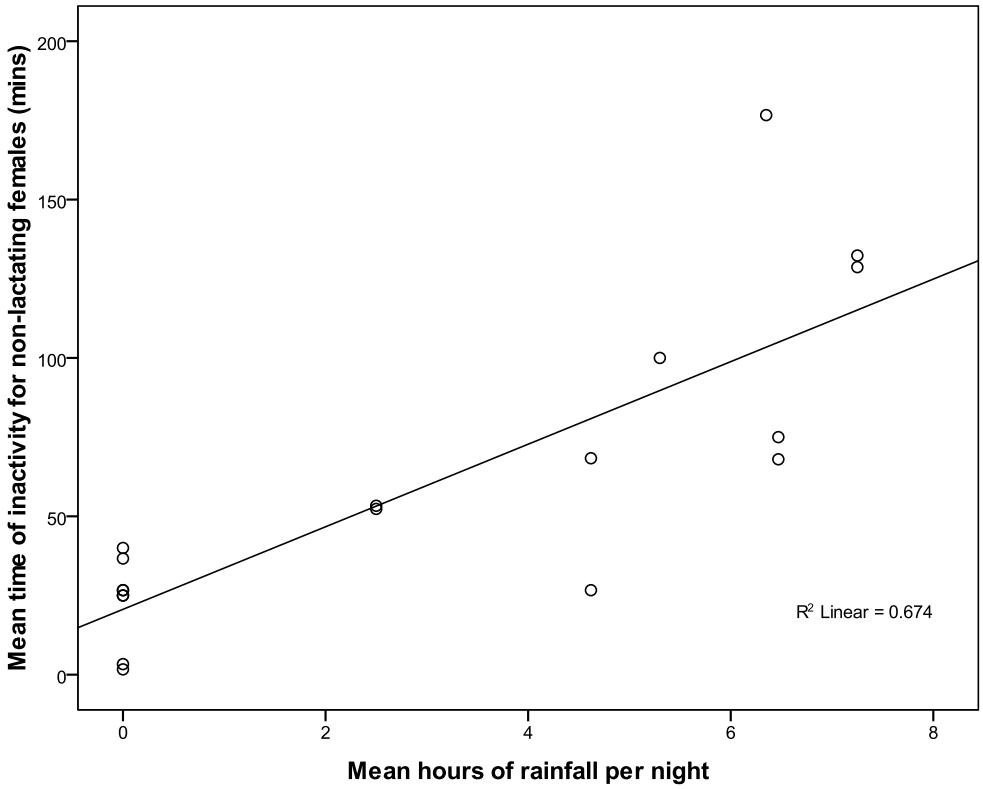


Figure 5.17a: Mean time spent inactive by radio-tagged non-lactating females was significantly correlated with the mean number of hours of rainfall per night.

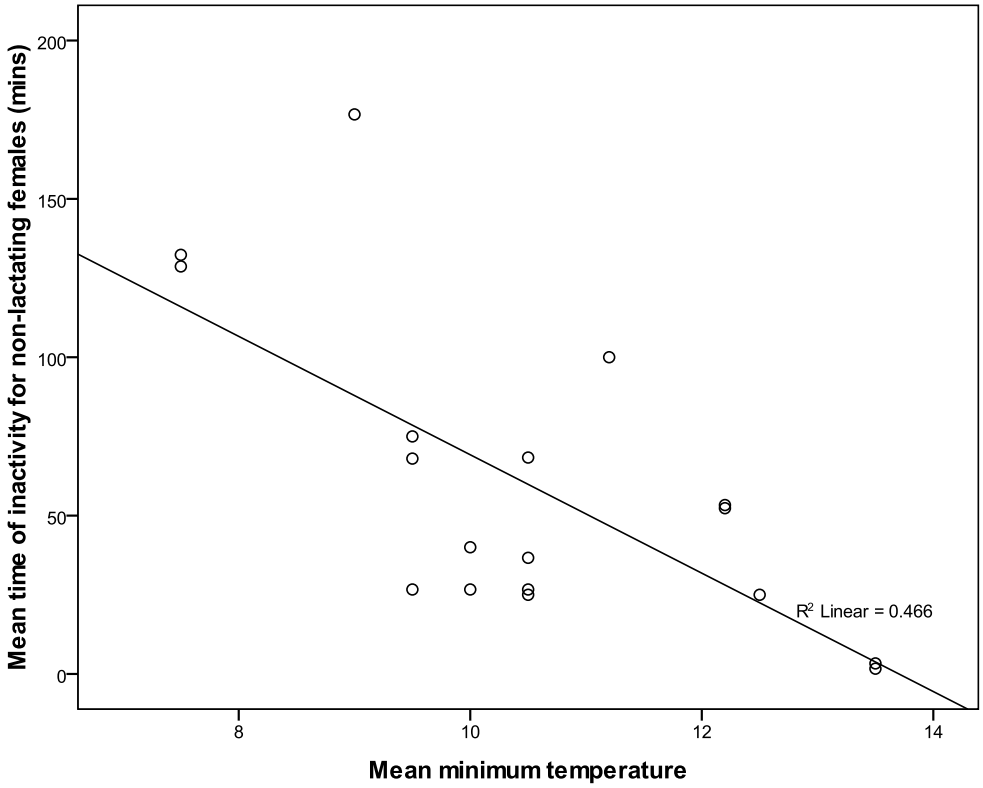


Figure 5.17b: Mean time spent inactive by radio-tagged non-lactating females was negatively correlated with the mean minimum temperature per night.

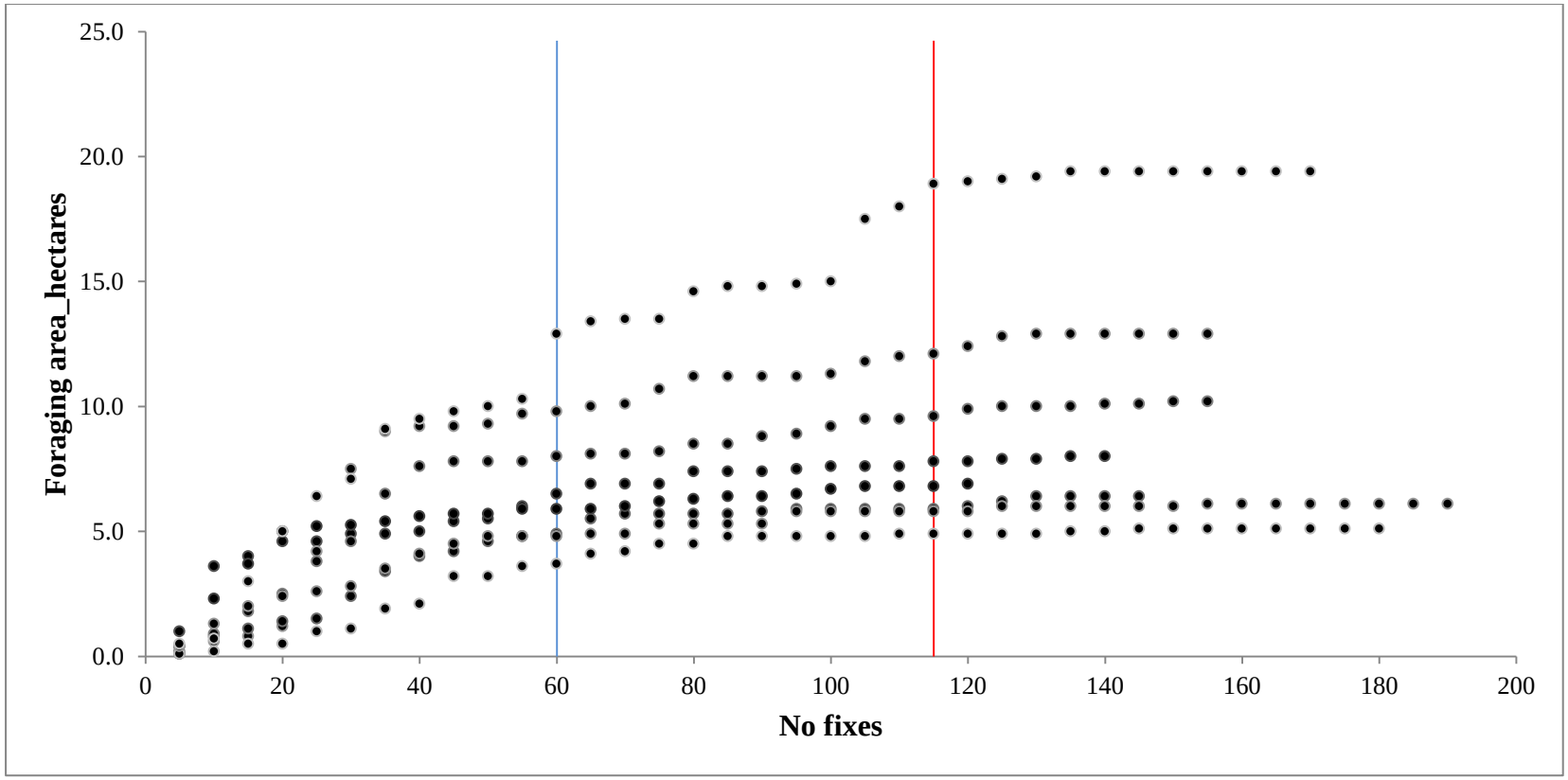


Figure 5.18: Cumulative home range size against number of successive locations of all female *P. auritus* radio-tracked in May – Sept 2007 (B8 – B15). The blue line shows the number of fixes whereby asymptote begins to start levelling off (ca. 60 fixes) and the red line indicates where asymptote begins to reach a stable estimate (ca. 115 fixes). Estimates of range size are based on 100% minimum convex polygons.

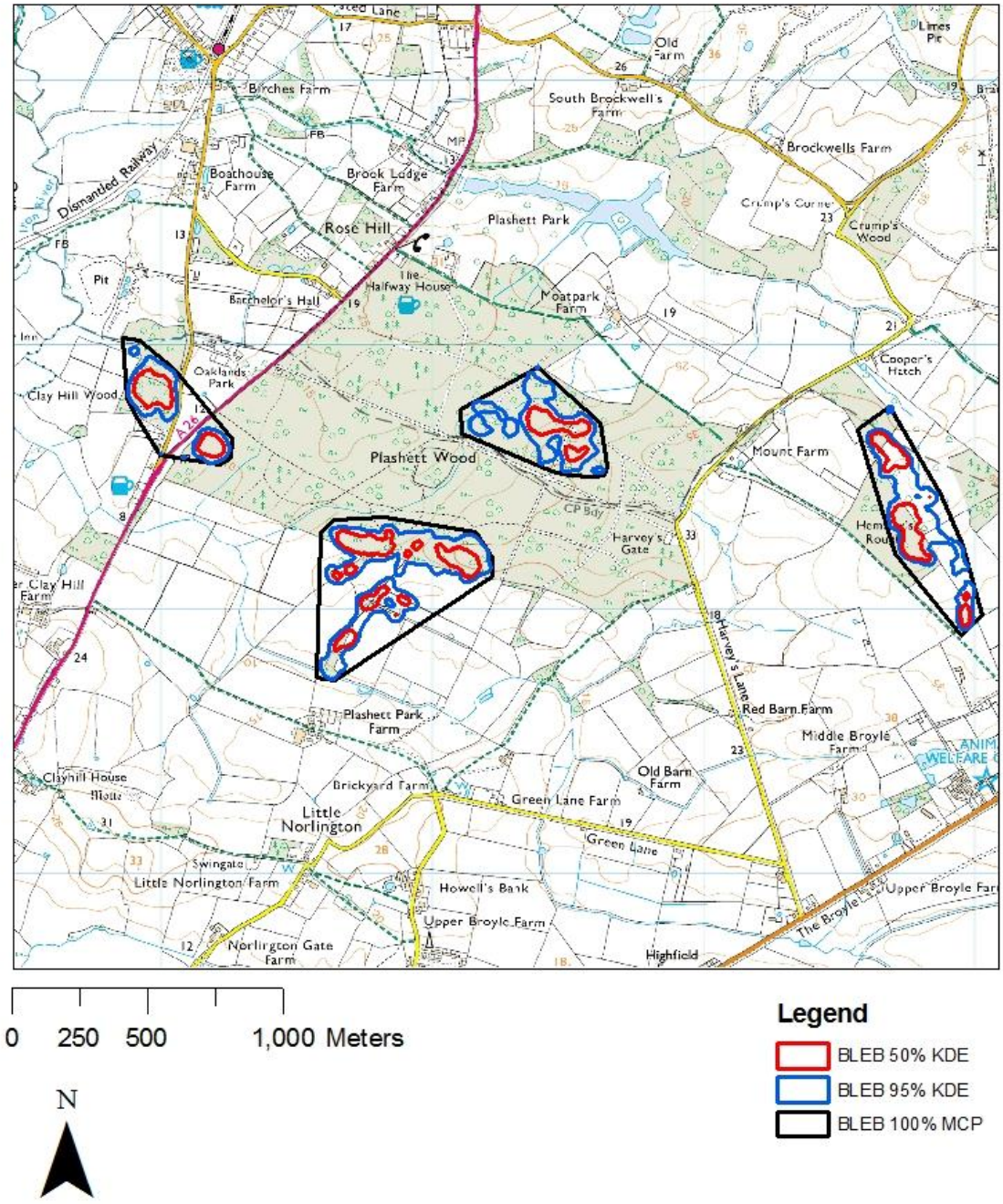


Figure 5.19: Spatial distribution of four individually radio-tracked bats in the Plashett Wood area in 2007. Individual home ranges are expressed as 100% MCPs enclosing all accurately recorded locations recorded. 95% KDE and 50% KDE reflect the distribution of the fixes, which are predominately associated with broadleaved woodland, copses and hedgerows.

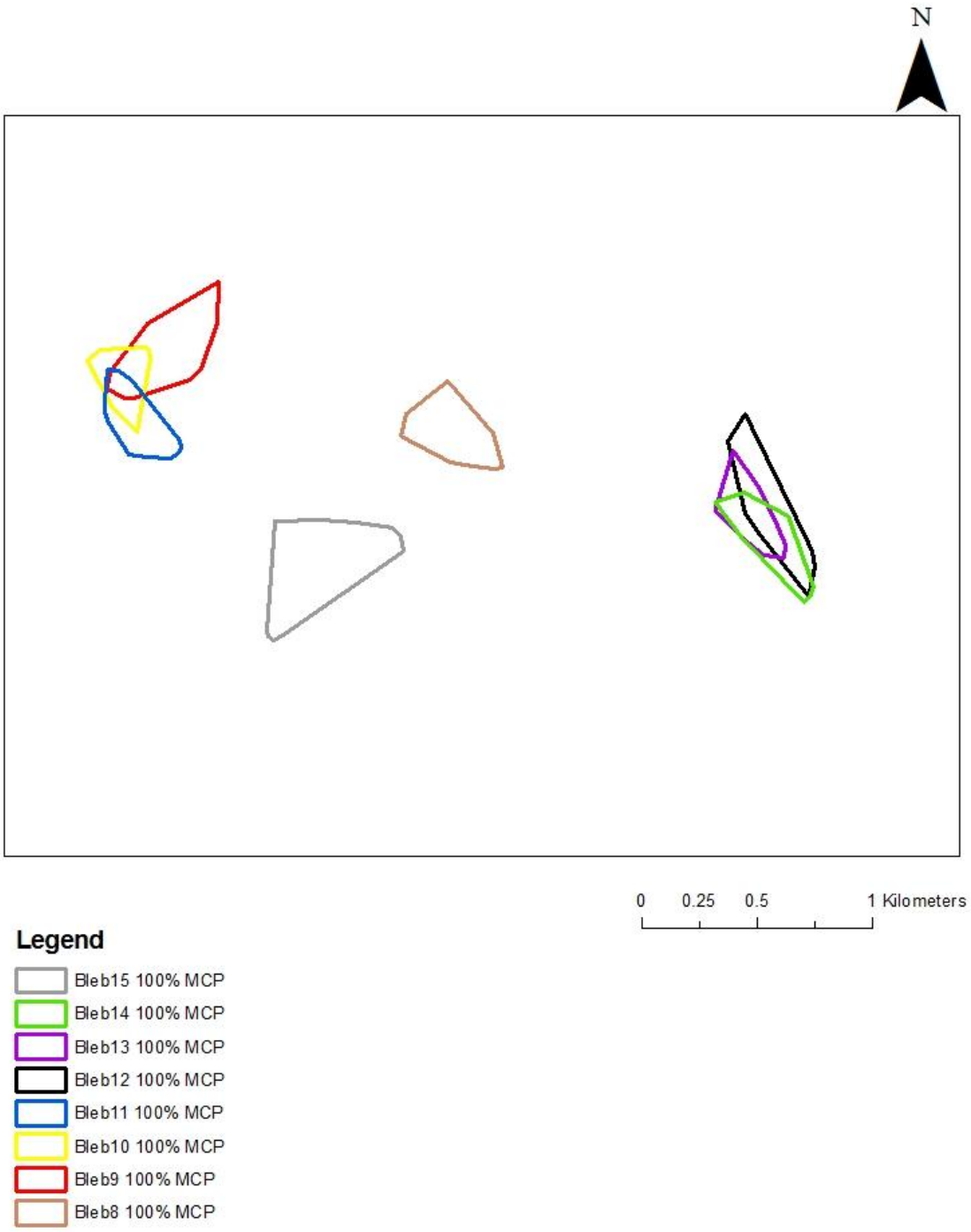


Figure 5.20: 100% MCP home range representation of eight females radio-tracked at Plashett Wood in 2007.

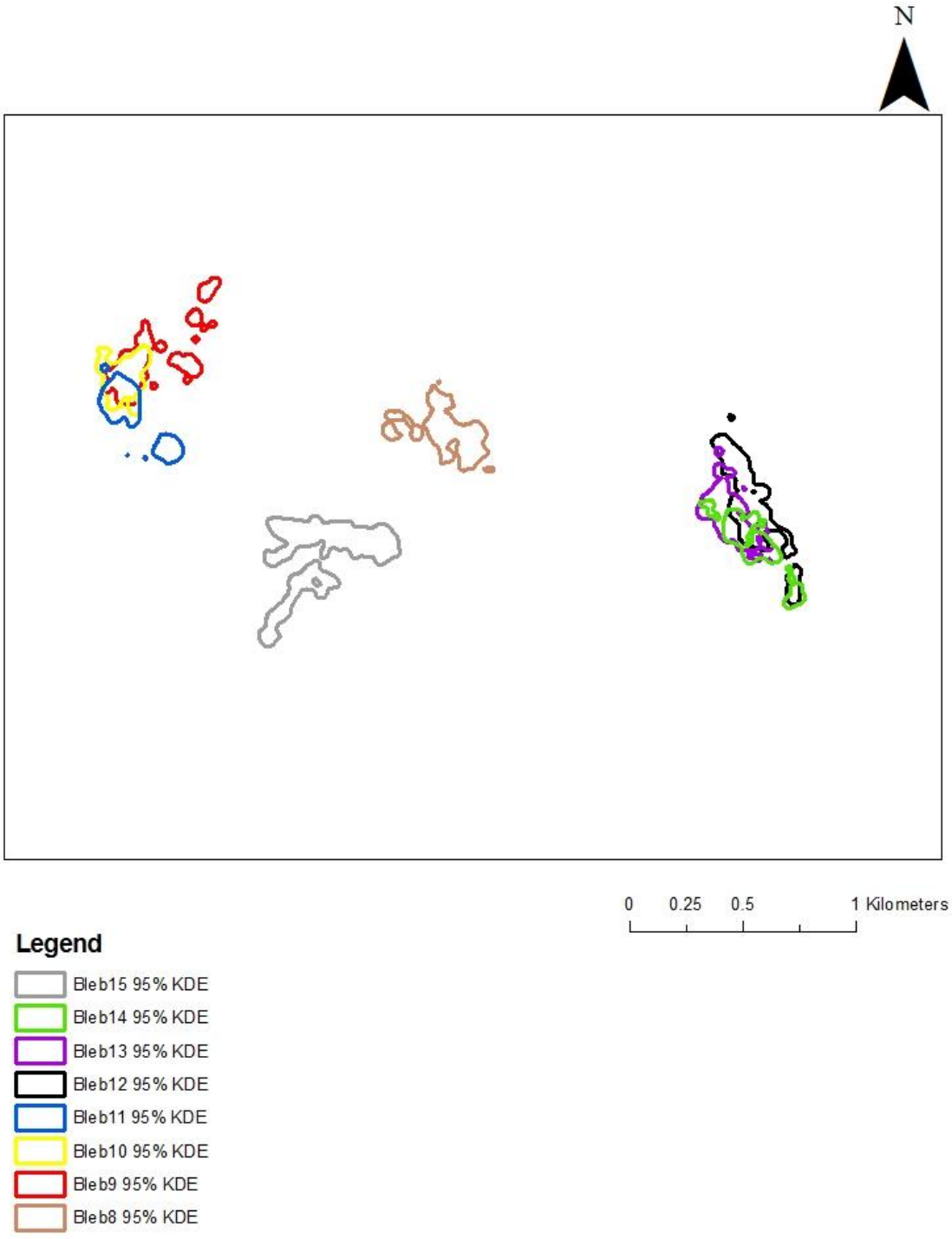


Figure 5.21: 95% KDE home range representation of eight females radio-tracked at Plashett Wood in 2007.

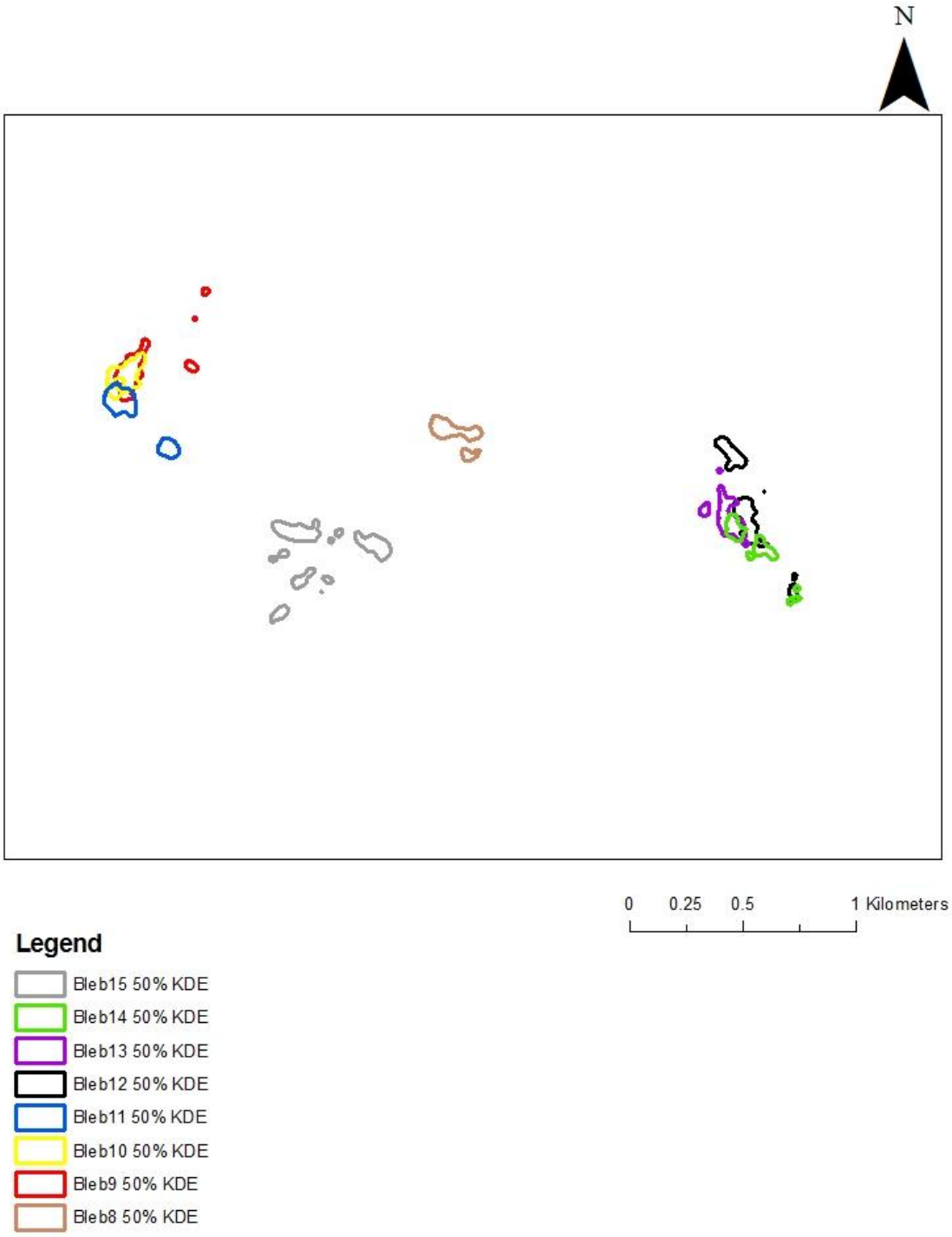


Figure 5.22: 50% KDE core foraging areas representation of eight females radio-tracked at Plashett Wood in 2007.

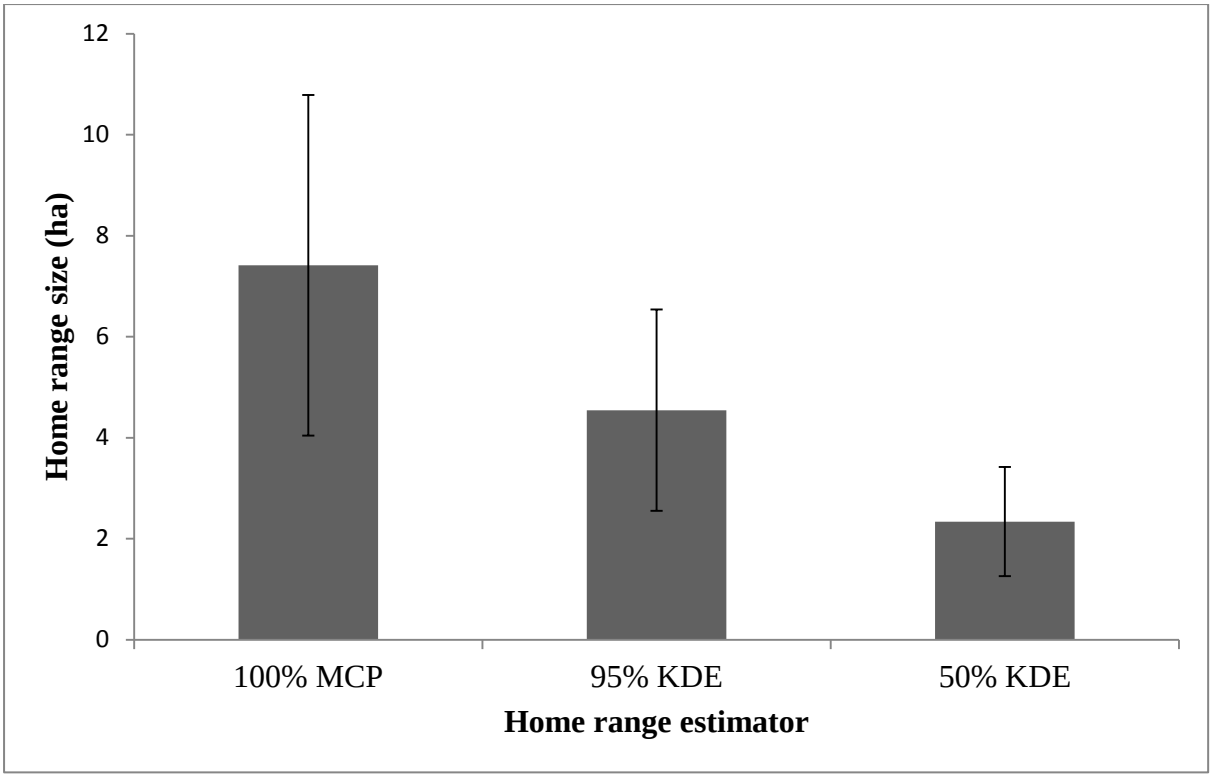


Figure 5.23: Mean ± standard deviation of home range size for 24 female *P.auritus*, radio-tracked from May – Sept 2007 and 2008. Estimates of range size are based on 100% minimum convex polygons, 95% & 50% kernel density estimators.

6.1 - Introduction

Territoriality as an attempt to maintain exclusive access to resources is a widespread phenomenon found in many animal species. In most species, however, actual fighting occurs far less frequently than one might expect from the occurrence of conflicts over territories and resources (Maynard-Smith and Harper, 2003). In mammalian behavioural ecology it is widely accepted that males compete for females (Dobson, 1982; Wolff, 1994) and females compete for resources such as food and shelter. Competition between female mammals for resources, other than mates, can have important reproductive consequences (Stockley and Bro-Jørgensen, 2010). The high energetic costs of gestation and lactation inherent in mammals (Loudon and Racey, 1987) means that the reproductive success of female mammals may be limited by access to resources such as food (Sterck et al., 1997; Stockley and Bro-Jørgensen, 2010) or nest sites (Hurst, 1987).

However, it has also been argued that female small mammals are territorial during the time of greatest food abundance and not during food limitation, which contradicts defence of resources hypotheses (Wolff, 1993; Wolff and Peterson, 1998). In which case alternative hypotheses, such as offspring defence, may better explain female territoriality in small mammals (Agrell et al., 1998). Not all female mammal species display territorial tendencies. Female territoriality is prominent among rodents, carnivores, insectivores, and some lagomorphs, but has been reported less frequently for bats, ungulates, marine mammals, marsupials, and most primates (Wolff and Peterson, 1998). It has been argued that in bats territoriality is extremely unlikely to occur in species that rely on a patchy sources of food that undergo large seasonal or unpredictable changes (Bradbury, 1977) and, in such

circumstances, group foraging may be a more optimal strategy. Nonetheless, it has been found that for other species of bats there is a tendency for females to use small individual foraging areas in pregnancy and lactation, for example in species such as Daubenton's bat *Myotis daubentonii* (Dietz and Kalko, 2007) and Bechstein's bat *Myotis bechsteinii* (Kerth et al., 2001; Dietz and Pir, 2009), and it is entirely plausible to suggest that some mechanism must exist in order for females to maintain their access to such a crucial resource, particularly when energy demands are at their greatest.

One mechanism for repelling intruders from a feeding territory is the advertisement of ownership by means of vocal communication (McComb and Reby, 2009). However, unlike species that emit social calls regularly at foraging sites, such as pipistrelle bats (Barlow and Jones, 1997; Russo and Jones, 1999), the emission of social calls away from the roost is a sporadic and infrequent event for *P. auritus*. The actually frequency is unknown, but it seems likely from continuous recording that bats typically call only a few times per night. Given this low frequency it would be extremely difficult to study the function of social calls by field observation and recording alone. Therefore, as an alternative approach, it was decided to investigate aspects of the function of social calls at foraging sites by examining how bats respond to playback rather than trying to investigate the conditions under which they are emitted).

There have been a number of functions proposed for the social calls of bats, given in differing contexts away from the roost, including: (i) defence of resources in pipistrelle bats (Racey and Swift, 1985; Barlow and Jones, 1997); (ii) attraction of mates in little brown bats *Myotis lucifugus* (Barclay and Thomas, 1979) and greater sac-winged bats, *Saccopteryx bilineata*

(Behr and von Helversen, 2004); (iii) group cohesion in spear-nosed bats *Phyllostomus hastatus* (Wilkinson and Boughman, 1998).

Chapter 4 detailed how *P. auritus* bats respond to synthesised social calls by rapidly approaching the source. Male and female *P. auritus* were caught using simulated social calls throughout the year, not only in the mating period, so the mate attraction hypothesis seems not to account for why *P. auritus* responded to the simulated social calls. The group cohesion hypothesis also seems unlikely to explain why individuals responded, as the response was often rapid and would sometimes appear aggressive (see Chapter 4). Therefore, defence of resources would appear to be a more plausible alternative explanation and, if true, it would be expected that there would be a differential response in relation to where the stimulus is located within a bat's foraging range. If a female *P. auritus* response to the stimulus represents defence of resources within their foraging territory, it would be expected that a female would be more responsive to the stimulus within her foraging range than outside of it.

Chapter 5 detailed how the home range for individual females was defined. Radio-telemetry provides a technique of tracking known individuals and providing estimates of location. However, ascertaining whether the focal radio-tagged bat has or has not responded to the stimulus is more problematic. This is because the transmitters are not always visible on infra-red cameras due to their small size and the fast fluttering flight of bats. Using telemetry to estimate the bat's position in relation to the stimulus would not provide enough rapid or precise data, especially considering the way in which *P. auritus* respond to the stimulus as

detailed in Chapter 4 (often a quick short responses). Therefore, in order to accurately identify the focal individual responding to the stimulus, it was essential to develop and test novel research equipment. An estimate of the distance from which *P. auritus* reliably responded to the stimulus was also required.

Section 6.2 details preparatory work in which three studies were conducted in order to develop and test novel research equipment. The first study describes the development of a system to register a close response of a radio-tagged individual, termed the 'LocateABat' system. The second study tests whether radio-tagged bats responded to the stimulus and, if so, whether the 'LocateABat' system registered an appropriate response. The third study describes a short experiment with captive bats to test their ability to hear the stimulus at differing distances under field conditions. Section 6.3 describes an experiment to test the hypothesis that a female's response to the stimulus represents defence of resources in relation to range use.

6.2: Preparatory Work & Pilot Studies

6.2.1: 'LocateABat' System

6.2.1.1 - Introduction

A system to detect when a radio-tagged bat approached the stimulus was designed and custom-built by Peter Reed of the University of Sussex. This detection system, termed 'LocateABat', was designed to sense when the signal of the radio-tag attached to the focal bat was within a given range. If the focal bat responded to the stimulus produced by the Autobat by approaching, then the 'LocateABat' system should also register a near response.

6.2.1.2 - Methods

6.2.1.2.1 - The LocateABat system design

An omni-directional antenna was attached to a Sika receiver that was set to the frequency of the radio-transmitter. The audio output of the Sika receiver was fed to a microprocessor-based circuit that measured the signal strength and converted this to a numerical value. This was output from the circuit via an RS232 serial data link to a laptop running a Visual Basic program that then displayed this value as a number on the screen. The value was also written to a file on the laptop along with the current real time. These values increased as the radio-transmitter approached the omni-directional aerial.

6.2.1.2.2 - System calibration

The output of the receiver was calibrated to produce five volts from the interface box when the transmitter was two metres away. The software on the laptop measured this voltage and displayed it as a reading (where 5V equated to a reading of 1000). As the radio-transmitter moved away from the antenna, the signal level dropped, the five volts dropped, and the value of 1000 dropped, proportionally. A field test was conducted to determine whether the 5V value of the radio-transmitter (which was set at two metres from the omni–directional aerial) dropped proportionately with increasing distance. The accuracy of the readings was dependent upon the proximity of the receiver to the transmitter, and this affected the accuracy of the estimates. Furthermore, topographical features or buildings commonly deflected signals or caused other interference (Priede, 1992; Parker et al., 1996) which would also influence the accuracy of the estimates.

Therefore, two experimenters (Stephanie Murphy and Peter Reed) tested the accuracy of the system in an open field (where topographical features or buildings were unlikely to interfere with the accuracy). A radio-transmitter was clipped to a piece of rubber tube and the tube was held by the first experimenter (to avoid the transmitter signal being affected by human contact). The first experimenter stood at a distance of two metres, at 180^{o}, from the omni directional aerial, and the volt meter (Tenma model 72-6260 Digital multi-meter) was set at 5 volts DC by the second experimenter. The first experimenter successively moved to a distance of 5, 10, 15, 20 and 25 metres away at 180^{o}, and the voltage registered on the volt meter was recorded by the second experimenter at each interval. This process was repeated a further twenty times in the opposite direction to ensure that the measures that were taken were comparative in different directions. The entire process was repeated in broadleaved deciduous woodland, the habitat type most frequently associated with *P. auritus* foraging (Swift and Racey, 1983; Entwistle et al., 1996); Murphy *et al* in prep.), in order to ascertain whether the system could produce reliable estimates of distance in the common foraging habitat of *P. auritus*. The consistency of the output was analysed using test/retest reliability (which calculates the variability of the resulting outputs) to compare the results of each test. The correlation coefficient between sets of results was used as a quantitative measure of the test-retest reliability using SPSS 17.0 (©SPSS Inc).

6.2.1.3 - Results

The results of the field trial studies revealed that the LocateABat system detects with 95% confidence when the radio-tag is within 10 metres of the omni directional aerial within broadleaved woodland (i.e. cluttered habitats as detailed in Table 6.1). Distances in excess of

10m were unlikely to be reliable, however, as there was too much variation from the readings registered, as indicated by the weak correlation between the test and the re-test. The accuracy of the detection rate improves in open areas, as detailed in Table 6.2, but as the experiments for testing *P. auritus* responses to the Autobat were to be carried out in broadleaved woodland, a conservative estimate of 10 metres was applied.

6.2.1.4 - Conclusions

The results of the pilot study, testing the accuracy of the LocateABat system, indicated that the methodology would be functional in detecting a radio-transmitter on a radio-tagged bat at a short range distance of up to 10 metres from the receiver.

6.2.2 – Trialling the LocateABat system

6.2.2.1 – Introduction

Once the LocateABat system had been developed and field tested using radio-transmitters, it was vital to ascertain whether the system would accurately register the radio-transmitters once attached to a bat. Moreover, it was essential to determine whether *P. auritus* would continue to respond to the stimulus once they had been radio-tagged (as the previous encounter with the stimulus had resulted in the individual being trapped, handled and a radio-transmitter attached, a negative experience that may induce 'response shyness'). Studies on vertebrates, such as the snapper fish, *Pagrus auratus*, found evidence that tagging induced trap shyness and tagged fish had a reduced probability of recapture by the method by which

they had originally been caught (Gilbert et al., 2001). Similarly, camera trapping studies on tigers, *Panthera tigris*, in India found that some individuals became trap shy (probably as a result of being scared by the flash) and capture rates decreased by more than 50% after the first five days of trapping. Studies on avian biology have also found that mist nets can be particularly inefficient in recapturing previously captured birds (Macarthu.Rh and Macarthu.At, 1974; Macarthur and Macarthur, 1974). However, not all vertebrates actively avoid traps as studies on badgers, *Meles meles*, have shown that some individuals are positively oriented toward being trapped (Tuyttens et al., 1999). The trappability of North American red squirrels *Tamiasciurus hudsonicus* was context dependent and positively correlated with their activity levels. A propensity for the squirrels to enter traps was associated with increased activity, intrusion on the territories of others, and moving farther from their own territory (Boon et al., 2008).

Therefore, the objectives of this study were to: (i) assess the practicality of the Locatabat system when used with transmitters on free-flying bats; (ii) ascertain whether radio-tagged bats, previously captured and tagged using the Autobat acoustic lure, would respond again; and, also (iii) test the feasibility of altering the stimulus location in relation to home range use.

6.2.2.2 - Methods

Six bats (five female and one male) were radio-tracked and their subsequent estimates of location analysed using the harmonic mean (HM) methodology (see Chapter 5 Section 5.2 for detailed radio-tracking study). For each bat, once sufficient data had been gathered to determine its range use over three successive nights, the experiment was set up in the 40% HM core foraging range. Data for two bats (one male, bleb 5, and one female, bleb 7) did not reach asymptote, indicating sufficient data had not been collected. However, it was decided to terminate radio-tracking to enable the trial experiment to commence (this being the primary focus of the pilot experiment). Poor weather conditions meant that extended periods of inactivity for these individuals occurred and there was the potential for the radio-transmitter to fail prior to the trial experiment being completed.

6.2.2.2.1 - Location of stimulus

The location chosen for the stimulus to be situated in the core area was within the area visited most frequently during the radio-tracking period, in the 40% HM. The location chosen for the stimulus to be situated within the peripheral foraging area (but excluding the 40% HM) was based upon locations visited less frequently (but nonetheless frequented) during the tracking period. In order to test whether the bats would respond to the stimulus outside of the previously determined home range, a location was chosen for the stimulus, in suitable *P. auritus* habitat, adjacent to the each focal bat's foraging range, but in a location at which they had not been previously recorded.

6.2.2.2.2 - Trial experiment

The Autobat speakers were suspended from a horizontal tree branch, using hooks, at a height of approximately three metres. Two Sony Handycam (Model DCR – DVD91E) digital video camera recorders were set up to film the bats responses to the Autobat. One camera was placed directly underneath the speakers filming vertically and the second camera was position approximately three metres away from the speakers filming horizontally. The camera filming the horizontal view filmed the focal bats behaviour as it approached the stimulus, but was not a reliable estimate of how close the bat approached. For this, a combination of horizontal and vertical cameras was necessary to film the bat from two different angles. Two infra red lights (Model Envin micro 75L) were used to illuminate the area around the speakers. The omni-directional aerial of the LocateABat system was placed near to the speaker to detect the approaching radio-tagged bat. A synthesised social call, call A (see chapter 4), was then produced by the Autobat and the bats that responded to the call were captured on film. The radio-tagged bats that responded also registered on the LocateABat system. Their vocal responses were recorded using a Petterson D240x bat detector and a Sony portable MD recorder (Model MZ-RH710). The Autobat broadcasted call A for a period of five minutes with an interval of five minutes of silence. After a period of one hour the process was terminated and repeated at the periphery and the exterior of each bat's foraging range. The position of the bat within its range was estimated at least every five minutes during the experiment (where possible) by a surveyor taking estimates of locations by radio-tracking.

6.2.2.3 – Results

6.2.2.3.1 - Responses of radio-tagged P. auritus *to synthesised social calls*

All five female bats responded to the Autobat when played in their core foraging range (40%HM). The mean number of responses for each female was 2.2 per hour. Two female bats responded when the stimulus was played in the peripheral range, and one female bat responded when the stimulus was played outside of her range. All confirmed responses of radio-tagged bats were captured on film and the output of the LocateABat system registered a peak in voltage concurring with the time of the filmed response (meaning we could be 95% confident that it was the focal bat responding within the five metre range). The radio-tracked male bat did not respond to the Autobat in either his core or peripheral range nor outside of his foraging range.

6.2.2.3.2 - Estimates of the distances radio-tagged bats responded

The estimates of locations, obtained from the radio-tracking data during the experimental period, were calculated and the distance (to the nearest metre) from the stimulus location to each estimated bat location was calculated. Each of the five female bats that responded to the stimulus in their core foraging range were located a mean distance of 65 metres (minimum 30; maximum 115) from the stimulus when the experimental began. The mean estimated distance of the five females from the stimulus in the peripheral foraging range was 175 metres (minimum 65; maximum 280) and, at the exterior of the foraging range, 300 metres (minimum 85; maximum 450). Table 6.3 details the number of times each radio-tagged bat responded to the Autobat, how close each bat approached to the stimulus and, also, the mean estimated distance that each bat was located from the stimulus during the pilot study.

6.2.2.4 – Discussion

The individuals that responded were recorded on infra-red video and a simultaneous response registered on the LocateABat system, which demonstrated that the LocateABat system was satisfactory for registering close responses of free-flying bats to the stimulus. All five radio-tagged female bats responded to the stimulus when it was played in their core foraging range (40%HM), with a mean of 2.2 responses per hour. Two female bats responded when the stimulus was played in the peripheral range, and one female bat responded when the stimulus was played outside of her range. The number of individuals responding, and the fact that some individuals responded on more than one occasion, indicates that the previous experience of being captured and radio-tagged was unlikely to deter motivation to respond to the stimulus.

It was considered that the differences in the response of *P. auritus* to synthesised social calls may have been influenced by the position within the bat's foraging area (i.e. core or peripheral) that the stimulus was located. However, the experiment needed to be carried out in the core, peripheral and exterior foraging range of each bat at a distance where it was known that the focal bat could hear the stimulus. Only one of the six radio-tagged bats responded to calls from the exterior of their range. However, based on radio-tracking data, the mean distance of the pilot study bats from the stimulus in the exterior location during the experimental period, was approximately 300 metres (SD = 127, n=5). By comparison, the mean distance of the pilot study bats from the stimulus in the core location during the experimental period, was 67 metres (SD = 34, n = 5). In order to test whether an individual's response was influenced by where in their range the stimulus was broadcast, a comparable

distance from the focal bat to the stimulus was required for each range location. This was to ensure that a result of 'no response to the stimulus' would mean that the bat heard and ignored the stimulus, as opposed to the focal individual not hearing the stimulus and, as a consequence, not being motivated to respond. Further analytical work on the distances *P. auritus* could hear the stimulus from was required in order to construct a robust experimental system to test whether responses to synthesised social calls are influenced by where the stimulus is located within their home range.

6.2.3 – Determining the most reliable distance from which *P. auritus* are likely to respond to the stimulus.

6.2.3.1 – Introduction

Social systems largely depend on communication among group members and the receivers' ability to assess the position and identity of the signallers (Bradbury and Vehrencamp, 1998). For effective acoustic communication to occur, an emitted signal must reach a receiver with enough clarity to allow an appropriate behavioural decision to be made (Arch and Narins, 2008). High frequency sounds attenuate more rapidly with distance (Morton, 1975; Lawrence and Simmons, 1982; Romer and Lewald, 1992; Morton, 1998) and, therefore, it would be expected that animals engaging in long-distance communication, or communicating in cluttered environments, would concentrate their vocal efforts by using low frequency sounds to ensure that their calls have a higher probability of reaching their intended target.

Although studies have demonstrated supporting evidence for the importance of nocturnal interaction among potential mates or group members (Barclay, 1982; Boughman and Wilkinson, 1998; Wilkinson and Boughman, 1998; Rossiter et al., 2002; Kerth and Morf, 2004), few studies have investigated the distance from which bats can hear these low frequency social calls due to the inherent difficulties in obtaining this information. However, a recent study by Hoffman *et al.* (2007) on the distance from which Greater sac-winged bats *Saccopteryx bilineata* can detect the echolocation calls of conspecifics, has reported an estimated maximum detection distance of 38 metres for echolocation calls broadcast within the forest under the sound transmission conditions of their study site and for an assumed

signal detection threshold of 20 dB SPL (50 m for a threshold of 0 dB SPL) (Hoffmann et al., 2007).

A study on the hearing capacity of *P. auritus* by (Coles et al., 1989) recorded a neural audiogram from the inferior colliculus and found that the *P. auritus* has good auditory sensitivity (less than 10 dB SPL) in the range of 7 – 55 kHz, containing a region of extremely low thresholds between 8 – 20kHz. The most sensitive frequency reached a threshold of -23 dB SPL at 11.7kHz (Coles et al., 1989). At this level it was found that there was heightened response to very faint noise stimuli. For example, even a slight movement by the experimenter (such as breathing) would cause the neural units to fire rapidly (Coles et al., 1989). In the field and when flying, *P. auritus* occasionally uses a loud long-sweep call with a very shallow terminal FM, containing a prominent energy peak at 12kHz (Ahlen, 1981). This frequency corresponds with the most sensitive region reported by Coles et al. (1989) and was reported by both authors to be optimal for long distance communication in the field. However, no published data on the distance from which *P. auritus* detects these low frequency calls has been reported.

Due to the low frequency nature of the simulated *P. auritus* social calls used to elicit responses of radio-tagged *P. auritus*, (as described in Chapter 4) it would be expected that these calls would travel a considerable distance. Therefore, the objective of this experiment was to ascertain the maximum distance from which the simulated social calls would be detected by *P. auritus* under natural conditions (i.e. cluttered woodland environments).

6.2.3.2 – Methods

Eleven *P. auritus* bats (five ♂ and six ♀) were obtained from local bat hospitals. The individuals were long-term captive bats (individuals that could not be released back to the wild due to the nature of their long-term injuries and were kept in captivity for educational purposes). *P. auritus*, in general, can be maintained as captive species relatively successfully (Racey, 1970). The bats were kept individually in mesh cages (Exo Terra Flexarium's 143 Litre capacity measuring 80cm x 40cm x 40cm) for a period of three days. A cotton towel was placed on the inside and allowed to hang up to create a dark compartment for sleeping. Live mealworms (*Tenebrio* spp.) and water were provided. This allowed time for the bats to habituate to their new environment prior to carrying out the experiment and to monitor the behaviour of the individuals. Animal husbandry guidelines for maintenance of the captive individuals were adhered to, as recommended by (Wilson, 1988).

To test the responses of the bats to the Autobat stimulus, each bat was placed initially in the mesh cage inside a large (open-ended) barn. This was to test whether there was an initial response from each bat prior to carrying out the experiment outdoors. The speakers were placed 10 metres back from the Autobat and four synthesised calls were played for a period of one minute with a one minute period of silence in between each call. This was repeated for each of the 11 bats. The behaviour of the bats was noted by the observers and their vocalisations were recorded using a custom-built broadband automated ultrasound recorder.

Two of the 11 captive bats showed no apparent positive response to the calls, both responding by going into torpor and exhibiting signs of stress. These two individuals were returned to the bat hospital and not included in any further experiments. The nine remaining bats were subsequently taken to Plashett Wood and the experiment to determine the distance from which they optimally responded to the stimulus was subsequently conducted.

The experiment was conducted in daylight hours in broadleaved woodland (to prevent potential vocal responses from other bats, which would be active at night-time, confounding the results). The mesh cage with the focal bat was positioned in a sheltered location and natural light was prevented from entering the mesh cage by placing a heavy duty cotton towel on top of the mesh cage. A Sony Handycam (Model DCR – DVD91E) digital video camera recorder was set up to film the bat's responses. Their vocalisations were recorded using a custom-built broadband automated ultrasound recorder.

A distance of 200 metres from the captive bat was measured using a measuring wheel (Trumeter 5000). The Autobat and speaker were placed at an initial distance of 200 metres and once the bat was observed to be 'settled' in the sleeping compartment (i.e. not moving or vocalising as detailed in Figure 6.1), four synthesised *P. auritus* social calls of differing sound intensity (call A, B, C and D see Chapter 4) were broadcast, for a period of one minute each with a period of silence of one minute in between each call. This process was repeated a further 38 times at five metre intervals until the speakers and Autobat were finally at five metres from the captive bat. The time that call was produced was noted. This process was repeated for each of the nine bats. The recorded film was edited using Sony Picture Package™ and the edited film was analysed and the behavioural responses noted. The ultrasound recordings were transferred to a laptop and saved as WAV files. The sound analysis software Bat Sound – Sound Analysis Version 2.1 (Pettersson Elektronik AB, Sweden) was used for the analysis of ultrasound recordings.

6.2.3.3 – Results

Seven of the nine long - term captive bats responded to the Autobat either physically, vocally or both. Two bats (one male and one female) did not respond to the stimulus and appeared to be in torpor when the experiment had finished. Table 6.4 details the responses of each individual to the stimulus. Three bats (two males and one female) responded by continually flying around the cage, echolocating and producing social calls. Two bats (females) responded by continually flying around the cage echolocating but not producing social calls. Two bats (females) responded by physically moving their head in the direction of the stimulus (but not flying). One of these females produced social calls whilst moving her head and the other female moved her head and echolocated. The mean maximum response distance of the responding individuals was 80 m (Range 55m – 105m; SD 15.81, n = 9) and the mean minimum response distance was 7 m (Range 5m – 20m; SD 5.67, n = 9).

6.2.3.4 – Discussion

The experiment with captive bats revealed that the mean maximum response distance to the stimulus was 80 metres (maximum 105m; minimum 55m). Therefore, the lack of response to the stimulus in the trial experiment (Section 6.2.2) would suggest that the focal individuals may have not heard the stimulus (as the mean estimated distance from the focal bats to the Autobat was 300 metres) and, as a consequence, would not be motivated to respond. In order to avoid this scenario, it was essential that the stimulus was broadcast at a distance from which it would be heard by the target individual. Based on the radio-tracking study, experiment and captive bat response estimates, a maximum distance from the stimulus location to the bat, at the start of the experiment, should be no greater than 50 metres.

6.2.3.5 - Conclusions

Captive bats responded to the stimulus in broadleaved woodland at a mean distance of 80m (maximum 105m, minimum 55m). The implementation of a distance of 50m from the focal bat, at each position in the focal bats foraging range (i.e. core, peripheral or exterior), should ensure that the focal bat is close enough to be able to hear the stimulus.

6.3: Responses of females in relation to stimulus location

6.3.1 - Introduction

Once sufficient data had been gathered from each of the 24 females to determine their core and peripheral foraging areas (as detailed in Chapter 5, Section 5.3), an experiment in which synthesised social calls were played at different localities within each female's foraging range was carried out. This section describes the methods and results of this experiment and, also, analyses whether the 24 females followed a predicted pattern of behaviour in relation to the physical response categorisation and the responses to simulated call type, as indicated by the responses of 'non-tagged' bats (see Chapter 4).

6.3.2 – Methods

6.3.2.1 - Locating the focal female

The foraging range for each female was displayed graphically on an OS map. The field workers subsequently approached the focal female on foot and the area in which she currently presented was recorded using GPS and marked onto the map using the "homing-in" method of (White and Garrott, 1990). It was ascertained whether the focal female was in her 'core' (50% KDE), peripheral (areas encompassed within her 95% KDE but excluding areas within the 50% KDE) or if she was in a location previously not recorded. If there was any element of doubt as to her approximate location, or if she was moving rapidly within her range, the field work surveyors waited until she was no longer moving rapidly and her location could be determined more clearly. The starting location (core / peripheral / exterior) for each female was rotated randomly so that previous encounters with the stimulus were unlikely to affect the overall result outcome.

6.3.2.2 - Experimental set up and procedure

Once the starting location for each female was determined, and the focal female was deemed to be within 50m of the stimulus location (a distance we could be confident that focal individual would be likely to hear the stimulus, see Section 6.2.3), the experiment was set up using the equipment and methodology detailed in Section 6.2.2.2. In addition, the Ultrabat, an automated broadband ultrasound recorder, was used to record vocal responses as opposed to the manual recorder used in the pilot study. Figure 6.2 shows an illustration of the experimental set up. If the female had moved significantly during the experimental set up period (approximately 4 minutes), and was considered to be more than 50 meters away from the stimulus, the stimulus location was altered accordingly.

Six different synthesised social calls (both amplified and non-amplified) were produced by the Autobat over a period of one hour (see Chapter 4 Section 4.4.4 for a full description of the calls used). The bats that responded to the Autobat over the one hour period were recorded on video. If the focal female with the radio-tag responded to the Autobat (as opposed to other *P. auritus* individuals in that locality), the LocateABat software registered a response reading indicating that she was within 10 metres at the exact time as the response was recorded on film. After one hour, the experiment was terminated and the process was repeated in the other two range localities. When possible, the experiment for each female was to be completed during one evening. However, in practice, this was not always possible as it was often difficult to locate the bat in peripheral locations during the tracking period (as they visited these areas less often). Therefore, the experiment for some females was conducted over a two or three night period.

Simultaneous bearings for each focal female were recorded for one hour prior to the experiment starting (before stimulus broadcast) and, also, for a period of one hour after the experiment was terminated, in order to detect the focal female movement patterns to assess how, or if, the production of synthesised social calls altered the focal females' behaviour.

6.3.2.3 - Statistical analysis

A chi-square test was used to determine whether there was an association between stimulus location and the number of females responding. Scheirer-Ray-Hare tests were used to examine whether there were seasonal effects on both observed behaviour and simulated call type, or range location effects on both observed behaviour and simulated call type. The observed mean response rate per hour for radio-tagged females, in each behaviour category

and for stimulus call type, in relation to season (i.e. month) was tested for trends using a Jonckheere Terpstra trend test. The responses for each call type were ordered in a manner such that the expected the medians of the group were predicted to change (using the data from the 'non-tagged' bats seasonal responses, chapter 4). So, for example, the order of the mean response per hour for call A of radio-tagged females in each month was compared to the order of the mean response per hour for call A of 'non tagged' bats in each month, in order to assess whether the medians of the groups ascend or descend in the same order. The estimated distance of the radio-tagged bat from the stimulus location during the experimental period was examined using regression analysis. All statistical analyses were carried out using SPSS 16.0 ©SPSS Inc.

6.3.3 - Results

6.3.3.1 - Responses of females to synthesised social calls in relation to range use

The number of individuals that responded to the stimulus and did not respond to the stimulus at different test locations is shown in Figure 6.3. In total, 20 females responded to the stimulus calls produced by the Autobat in their core foraging area (50% KDE) and four females did not respond. Twelve females responded to the stimulus calls produced by the Autobat in the peripheral foraging area (95% KDE excluding 50% KDE) and 12 females did not respond. Five females responded to the stimulus calls produced by the Autobat outside of their foraging range (exterior to 95% KDE) and 19 did not respond to the calls. Table 6.5 details the individuals that responded, the number of responses at each locality, and the females that produced a vocal response. Two females did not respond at all. A chi-sq test

demonstrated that there was a significant association between stimulus location and the number of individuals responding (X^2 = 18.79, d.f. = 2, p < 0.0001).

There was evidence of extensive overlap in the foraging areas of females (investigated further in Chapter 7). This raises the possibility that some individuals may have been preconditioned to the stimulus when it was played for a female whose range overlapped with their own. When data for these eight potentially 'pre-conditioned' females were excluded from the analysis, a subsequent chi-sq test demonstrated that there was still a significant association between stimulus location and the number of individuals responding (X^2 = 12.69, d.f. = 2, p < 0.0017).

Of the 20 females that responded to the stimulus in their core foraging area, 14 responded on more than one occasion (mean = 2.2 responses, SD = 1.15). Of the 12 females that responded to the stimulus in the peripheral foraging area, three responded on more than one occasion (mean = 1.5 responses, SD = 1.16), shown in Figure 6.4. Of the five females that responded to the stimulus outside of their foraging ranges, only one responded on more than one occasion (mean = 1.2 responses, SD = .44). The mean number of times a responding female approached the stimulus in the core foraging area was significantly greater than the mean number of times of approach in the peripheral foraging area or external to the foraging areas (Kruskal-Wallis ANOVA H = 7.466, n = 37, p < 0.024). There was no evidence of either a seasonal effect in the number of responses to the stimulus F (1, 4) = 1.156, p = 0.340 or evidence of an interaction between response in relation to season and range F (1, 8) = 0.504, p = 0.848.

6.3.3.2 - Vocal responses of radio-tagged females to the stimulus

Of the 20 females that responded to the stimulus in their core foraging areas, a vocal response (i.e. social call) was recorded for only nine responding females. A vocal response was recorded for six of the 12 females in the peripheral foraging area and in the exterior foraging area a vocal response was recorded for two of the five responding females. There was no significant difference between the numbers of individuals responding vocally in the core, peripheral or exterior foraging ranges (Kruskal-Wallis ANOVA $H = .154$, $n = 37$, $p < 0.926$).

6.3.3.3 - Behavioural responses of radio-tagged females responding to the stimulus

A total of 81 hours producing synthesised calls resulted in a total of 68 responses to the stimulus from 22 of the 24 radio-tagged females. The 68 responses could be categorised into six of the seven behaviour categories (categories one to six as detailed in Figure 4.16, Chapter 4). There was a significant difference in the mean response rate per hour between categories (Kruskal-Wallis ANOVA $H = 17.818$, $df = 6$, $p = 0.0007$) as shown in Figure 6.5. However, when behaviour seven (unclassified behaviour) was excluded from the analysis no significant difference was found (Kruskal-Wallis ANOVA $H = 7.378$, $df = 5$, $p = 0.194$). Excluding behaviour seven, a Scheirer-Ray-Hare test found that there was also no evidence of a significant difference in response rate per hour between month, behavioural response types or evidence of a significant interaction between month and behavioural response type (test statistics detailed in Table 6.6).

A Scheirer-Ray-Hare test found that there was a significant difference in the mean response rate per hour for radio-tagged females at each range location (i.e. core, peripheral, exterior) but no significant differences in behavioural response types or evidence of an interaction between range location and behavioural response (test statistics detailed in Table 6.7). There were significantly more behavioural responses in the core range compared to the exterior

(Mann U Whitney Z = - 4.059, n = 287, p > 0.0001) and significantly more behavioural responses in the peripheral range compared to the exterior (Mann U Whitney Z = - 2.379, n = 288, p = 0.17), but no significant difference in the number of behavioural responses between the core and the peripheral ranges (Mann U Whitney Z = - 1.907, n = 8289, p = 0.056).

6.3.3.4 – Behavioural responses of radio-tagged females to synthesised social calls (stimuli) of varying intensity.

There was a significant difference in the effectiveness of the call types in eliciting the 68 responses from radio-tagged females (Kruskal-Wallis ANOVA H = 17.818, df = 6, p = 0.022; as shown in Figure 6.6). When the responses from when the stimulus was silent are removed from the analysis, there was still a significant difference in the effectiveness of the call types (Kruskal-Wallis $_{ANOVA}$ H = 11.745, df = 5, p = 0.038). Table 6.8 details the post hoc analysis (using Mann U Whitney with a Bonferroni correction to reduce Type 1 error rate (.05/7) resulting in a critical value for significance of 0.007) to investigate where the differences between call types were significant. Call A was significantly more effective at eliciting behavioural responses to the stimulus compared to call B, but no other significant differences were found in the effectiveness of calls for radio-tagged females (test statistics for pair-wise comparisons detailed in Table 6.8).

A Scheirer-Ray-Hare test found no significant difference between month, call types, or a significant interaction between month and call type for radio-tagged females (test statistics detailed in Table 6.9). There was a significant difference in the mean response rate per hour of radio-tagged females at each range location (i.e. core, peripheral, exterior, as previously

detailed), but no significant differences in call type or evidence of an interaction between call type and range location (test statistics detailed in Table 6.10).

A Jonckheere's trend test was carried out to investigate whether there was a trend in the responses of the radio-tagged females in relation to the observed behavioural responses, using the order of the response categories from the data shown by all non–tagged responding *P. auritus* as a predictor for seasonal response (as detailed in Chapter 4). There was no evidence of a significant trend in the response categories for each month for the radio-tagged females (test statistics detailed in Table 6.11).

A Jonckheere's trend test was also carried out to investigate whether there was a seasonal trend in the response rate of tagged females to each call type that agreed with that found in non-tagged females (as detailed in Chapter 4). Figure 6.7 shows the mean response rate per hour of radio-tagged females to each call type in each month. The only calls that radio-tagged females responded to in May and June were calls A & D (high intensity high repetition) but there was no overall significance in the trend (test statistics detailed in Table 6.12). This is likely to be as a result of the large variation in the response rate per hour (indicated by the large error bars). However, there was a significant trend for Call C (low intensity high repetition), where responses to Call C were only observed for radio-tagged bats in August and September. There also appears to be a pattern whereby high intensity calls elicit responses earlier in the season (May – July), whereas, later in the season, all call types seem equally effective.

6.3.3.5 - Time of response

Each time the stimulus was broadcast, regardless of location in relation to foraging area, the focal female was always within 50 metres of the stimulus. Most responses of radio-tagged females occurred within the first fifteen minutes of the stimulus broadcast, as shown in Figure 6.8, and the number of responses to the stimulus was negatively correlated with experimental time (Spearman rank correlation $r_s = -0.936$, $n = 12$ $p < 0.01$; Figure 6.8). The time of the first response in relation to stimulus location for radio-tagged females was also negatively correlated with stimulus broadcast time in the core foraging areas (Spearman rank correlation $r_s = -0.930$, $n = 12$, $p < 0.01$), negatively correlated (although weaker) with time of first response in the peripheral areas (Spearman rank correlation $r_s = -0.746$, $n = 12$, $p < 0.01$) and, also, outside the foraging area (Spearman rank correlation $r_s = -0.815$, $n = 12$, $p < 0.01$), as shown in Figure 6.9.

6.3.3.6 - Movement of females in their home range in relation to stimulus location

Estimates of location for each female were obtained when the stimulus was broadcast at each range location and for a one hour period after the stimulus had finished broadcasting. For the 22 females that responded, there appeared overall to be a general pattern that, once a female had approached the stimulus (presumably to investigate the source of the sound), her subsequent foraging behaviour was often at a distance of over 150 metres from the stimulus. Once the production of simulated social calls was terminated, the females returned to forage in closer proximity to the stimulus location, as shown for (Bleb 8), in Figure 6.10.

The number of fixes available for females for the hour after their experimental period varied between individuals, because it was not always possible to continue radio-tracking at fixed intervals at the same time as relocating equipment for the next experimental session.

However, a comparison of the time spent at each range location was analysed for each of the 20 females that responded to the stimulus in their core foraging area when the stimulus was broadcasting compared to when the stimulus had terminated broadcasting. Each estimated location of the focal female obtained during the experiment time (a one hour period) and after the experiment was finished (also for a one hour period) was assigned to be within the core, peripheral or exterior range). The proportion of fixes in each locality was converted to a percentage, so if ten fixes were recorded for a female, eight were in the core, two in the peripheral and none recorded outside the range, the proportions would be 80% core, 20% peripheral, and 0% outside known range. The females that responded to the simulated social calls in their core foraging range spent less time within their core foraging range when the Autobat was broadcasting compared to the time spent in the core foraging area when the Autobat had terminated broadcasting (Wilcoxon Signed Ranks $Z = -3.379$, $n = 20$, $p < 0.001$). There was no similar trend found for the females that responded in the peripheral foraging area (Wilcoxon Signed Ranks $Z = -.472$, $n = 12$, $p < 0.637$) or outside the foraging area (Wilcoxon Signed Ranks $Z = -.135$, $n = 5$, $p < 0.892$).

6.3.4 – Discussion

The main aim of the study was to investigate the response of female *P. auritus* to simulated social calls in relation to range use. Using the results of the radio-tracking data, detailed in Chapter 5 Section 5.2, the stimulus location was set up to within 50 metres of the focal female, in areas deemed to be within her core and peripheral foraging range and, also in an area outside of her range (where she had previously not been recorded in the radio-tracking study).

Females were more likely to respond to simulated social calls played in their core foraging range than in their peripheral foraging range or outside of their range. To date, no other studies have been carried out on bats where playback (or the use of synthesisers to produce simulated calls) has been used as a tool to investigate the responsiveness of individuals to the stimulus in relation to range use. However, similar studies have been undertaken with other animals. For example, a study on a wild population of Swift foxes *Vulpes velox* found that male territory holders responded more intensely with barking if a playback simulating intrusion by a rival occurred inside of the core (50% kernel contour isoline of estimated home range) compared to outside (Darden and Dabelsteen, 2008). The authors reported a high degree of overlap in the home range core by neighbouring home ranges (similar to *P. auritus* see Chapter 7) so territoriality exclusivity was unlikely to account for the increased intensity of response. In contrast, a study on Grey wolves *Canis lupus* using playback of simulated wolf howls found that the responses to simulated intrusions were independent of the location within the home range (Harrington and Mech, 1983). The propensity of *P. auritus* to respond maximally to simulated social calls in core foraging areas is consistent with the resource defence hypothesis. Repelling intruders from core foraging areas may reduce competition for resources within them.

Resource defence has been suggested as the underlying cause of aggressive interactions, characterised by behaviour such as chases and low frequency vocalisations, in feeding areas for species such as the hoary bat *Lasiurus cinerus* (Belwood, 1982; Fullard, 1982) and pipistrelle bats (Barlow and Jones, 1997) which, in turn, appeared to be associated with the abundance of prey (i.e. aggressive behaviours and social call production increase at low insect densities). For other bat species studied in the field in considerable detail, such as

Myotis lucifigus, (Fenton et al., 1976; Anthony and Kunz, 1977; Fenton and Bell, 1979; Aldridge et al., 1990), there has been no reported evidence of aggressive encounters between foraging individuals. However, these are species that use multiple feeding areas and not small areas of woodland like *P. auritus*.

Resources may also only be worth defending at particular times of year (e.g. birth and lactation when energy demands are greatest) or against particular types of individuals within the population (i.e. roost members versus strangers). In this study, however, there was no evidence of a significant difference in the number of responses for radio-tagged females in relation to season. Nonetheless, the time of first response to the stimulus, for the majority of females, tended to be prompt (i.e. most responses occurred within 15 minutes of the stimulus broadcast), indicating that perhaps the inclination to respond to the stimulus may be as a necessity to investigate unfamiliar ultrasound, which resemble intra-specific social calls, within their foraging range.

There was no evidence of a significant seasonal effect in call type for eliciting responses from radio-tagged females. However, only four females were tested in May and June, and all of their responses were to high intensity high repetition calls. In July, five females were tested and all of their responses were also to high intensity calls with both high and low repetition, whereas in August and September there was evidence of responsiveness to all call types. In heart-nosed bats *Cardioderma cor*, (Vaughan, 1976) reported a loud audible call with energy at 12kHz which is used to establish exclusive foraging areas. It may be that, earlier in the season, the propensity of *P. auritus* to respond maximally to loud high intensity calls indicates that these call types are used to establish vital foraging areas, close to roost sites, to

ensure successful reproduction at a critical time of year. There was also a significant trend in response to low intensity high repetition calls (such as call C) later in the season. The function of such a call type may could, for example, be related to mating or mate attraction. However, it would be necessary to test the responses of both males and females to confirm such a prediction. It could easily be that the function of different call types changes throughout the year, as demonstrated for various species of birds (Timcke and Bergmann, 1994; Lucas et al., 2007).

An alternative explanation to the resource defence hypothesis could be that the response to stimulus may represent attraction to resources. Responses to calls could represent co-ordination of foraging (i.e. attraction to food resources). Greater spear-nosed bats, *Phyllostomus hastatus*, are known to emit audible screech calls which appear to function as contact calls that recruit and co-ordinate foraging among group members (Wilkinson and Boughman, 1998). Food associated calls have also been demonstrated for other species, particularly primate species (Gros-Louis, 2004; Gros-Louis, 2006; Clay and Zuberbuhler, 2009; Slocombe et al., 2010). Advertising food sources to conspecifics may be beneficial to maintain a stable social group if food sources are in abundance (i.e. the benefits of sharing outweigh the costs). If *P. auritus* females response to the stimulus represents attraction to resources, then it would be expected that there would be some evidence of co-ordination of foraging between females. This is investigated further in Chapter 7.

Finally, on a practical note, the declining responsiveness of radio-tagged females may represent habituation to the stimulus over time, reducing its effectiveness for use as a lure. Once a radio-tagged female responded to the stimulus in her core foraging area, there was a tendency to move away from the stimulus to forage elsewhere whilst the stimulus continued

to broadcast. There was evidence that females spent less time in their core foraging areas when the stimulus was broadcasting for 60 minutes compared to the subsequent 60 minutes when the stimulus finished broadcasting. Therefore, there is the potential for the stimulus to alter female foraging behaviour, which, if used continuously at foraging sites, could affect the fitness of *P. auritus* females by deterring them from core feeding areas.

6.4 – Summary

- The experiment showed that female *Plecotus auritus* were significantly more likely to respond to a stimulus produced within their core foraging area, than in peripheral area or outside their foraging area.
- Female *Plecotus auritus* also approached the stimulus significantly more often in the core foraging area compared to the peripheral foraging area and external to the foraging area.
- There were no significant differences in behavioural response types and no evidence of an interaction between range location and behavioural response.
- The time of first response in relation to stimulus location for radio-tagged females was negatively correlated with stimulus broadcast time.

	Cluttered habitat field test 1			Cluttered habitat field test 2			Reliability Test - Retest	Reliability Test - Retest
Distance	**Mean open field reading (volts)**	**SD**	**LocateABat Output**	**Mean woodland reading (volts)**	**SD**	**LocateABat Output**	**Pearson Correlation**	**Significance**
2	4.5835	0.03014	1000 - 990	4.5815	0.02834	1000 - 990	0.474	**0.035**
5	2.8775	0.03401	989 – 927	2.8370	0.11563	989 – 927	0.523	**0.018**
10	1.8665	0.05566	926 – 642	1.8990	0.02594	926 – 642	0.453	**0.045**
15	0.9295	0.07156	641 - 501	0.9700	0.06087	641 - 501	-0.092	0.701
20	0.5895	0.14409	n/a	0.6640	0.21303	n/a	-0.287	0.220
25	0.4615	0.44683	n/a	0.3510	0.14160	n/a	-0.150	0.528

Table 6.1: LocateABat trial experiment results to test for accuracy of the system in cluttered environments. The volts reading indicate the reading on a voltmeter used in the field. The 'LocateABat' output details the range readings obtained from the system at differing distance intervals. The Pearson correlation coefficient for the total test-retest score of both tests at each measurement interval (and the significance of the results) is detailed.

	Open field test 1			Open field test 2			Reliability Test - Retest	Reliability Test - Retest
Distance	**Mean open field reading (volts)**	**SD**	**LocateABat Output**	**Mean woodland reading (volts)**	**SD**	**LocateABat Output**	**Pearson Correlation**	**Significance**
2	4.5880	0.03302	1000 - 990	4.5945	0.02665	1000 - 990	0.866	**0.010**
5	2.9350	0.03517	989 – 927	2.9425	0.04266	989 – 927	0.714	**0.010**
10	1.8710	0.04994	926 – 642	1.8945	0.03940	926 – 642	0.546	**0.013**
15	0.9375	0.05571	641 - 501	0.9420	0.06950	641 - 501	0.560	**0.010**
20	0.6110	0.12945	n/a	0.6905	0.21765	n/a	-0.115	0.631
25	0.3535	0.11860	n/a	0.3535	0.16096	n/a	0.367	0.112

Table 6.2: LocateABat trial experiment results to test for accuracy of the system in open environments. The volts reading indicate the reading on a voltmeter used in the field. The LocateABat output details the range readings obtained from the system at differing distance intervals. The Pearson correlation coefficient for the total test re-test score of both tests at each measurement interval (and the significance of the results) is detailed.

40% HM Core						**80%HM Peripheral**					**Exterior**				
Bat	Response	Number of responses	Estimate of how close bat approached (m)	Mean distance from stimulus	SD	Response	Number of responses	Estimate of how close bat approached (m)	Mean distance from stimulus	SD	Response	Number of responses	Estimate of how close bat approached (m)	Mean distance from stimulus	SD
2♀	Yes	2	1	55	41	No	n/a	240	281	36	No	n/a	364	448	94
3♀	Yes	1	2	113	75	No	n/a	129	185	34	No	n/a	183	299	92
4♀	Yes	3	1	92	123	Yes	1	2	185	138	No	n/a	213	270	33
5♂	No	0	55	251	142	No	n/a	93	261	246	No	n/a	287	303	14
6♀	Yes	3	1	31	26	No	n/a	39	65	18	No	n/a	318	402	71
7♀	Yes	2	1	42	39	Yes	1	1	73	43	Yes	1	2	83	49

Table 6.3: Response of radio-tagged *P. auritus* to Autobat and estimates of distances (based on triangulation) from stimulus, during playback in 40% HM (core), 80% (peripheral) and outside (exterior to MCP) foraging range.

Figure 6.1: *P. auritus* prior to the stimulus being played. The bat is settled in the sleeping compartment.

Bat no.	Sex	Date of expt.	Response to stimulus	Maximum response distance (m)	Minimum Response distance	Flight	Attentive	Social Calls	Echolocation
3	Male	03.5.07	Yes	90	5	✓	✓	✓	✓
4	Male	03.5.07	No	n/a	n/a	×	×	×	×
5	Male	05.5.07	Yes	75	5	✓	✓	✓	✓
6	Female	05.5.07	Yes	80	5	✓	✓	×	✓
7	Female	14.5.07	Yes	105	5	✓	✓	✓	✓
8	Female	14.5.07	No	n/a	n/a	×	×	×	×
9	Female	14.5.07	Yes	55	5	×	✓	✓	✓
10	Female	20.5.07	Yes	85	5	×	✓	×	✓
11	Female	20.5.07	Yes	70	20	✓	✓	×	✓

Table 6.4: Responses of captive *P. auritus* to simulated social calls, produced by the Autobat, in broadleaved woodland. The maximum and minimum distance each individual responded, along with the behaviour for each bat is detailed.

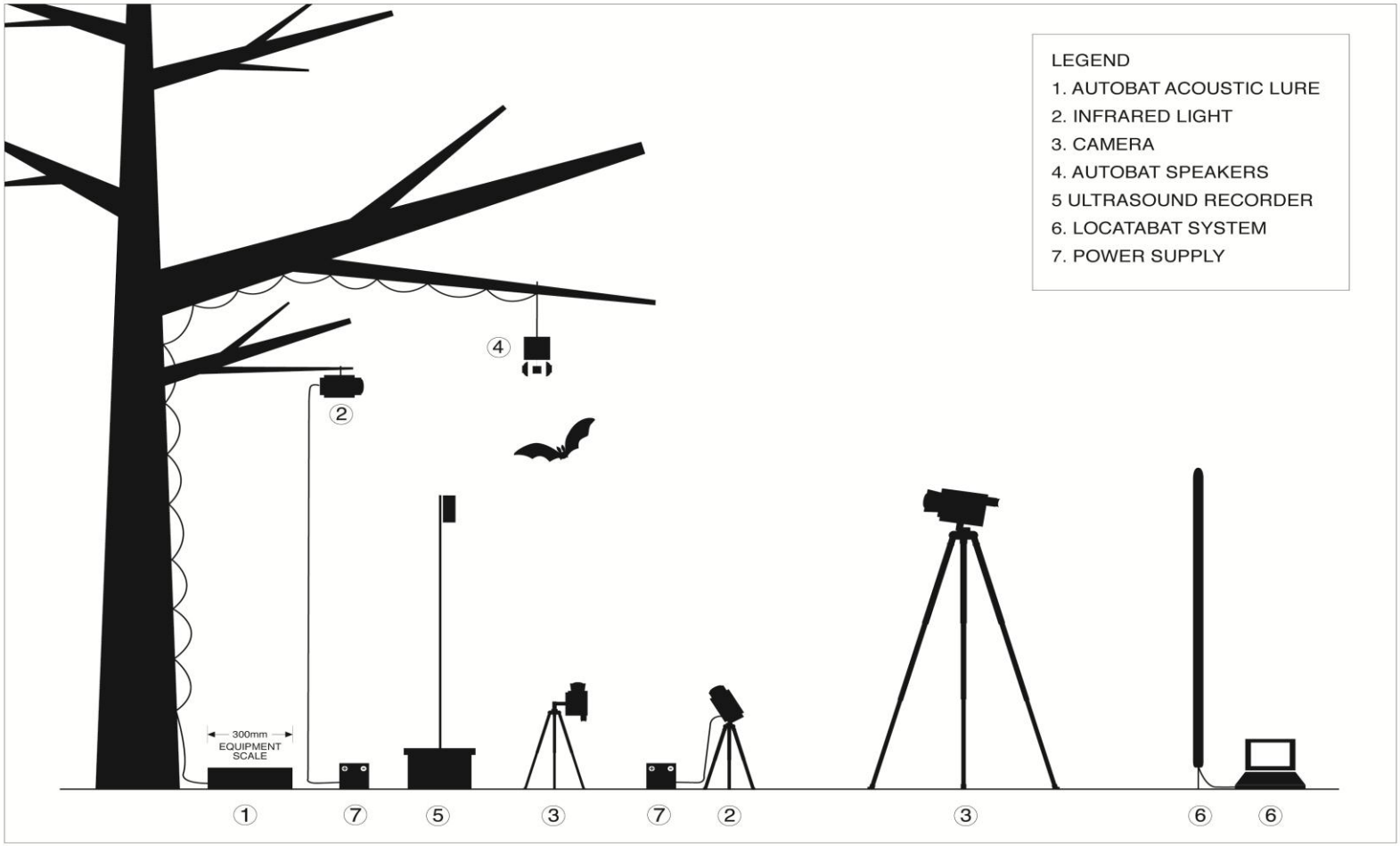


Figure 6.2: Positions of the equipment used in the LocateABat experiment to playback synthesised *P.auritus* social calls to radio-tagged females to assess their response in relation to range use.

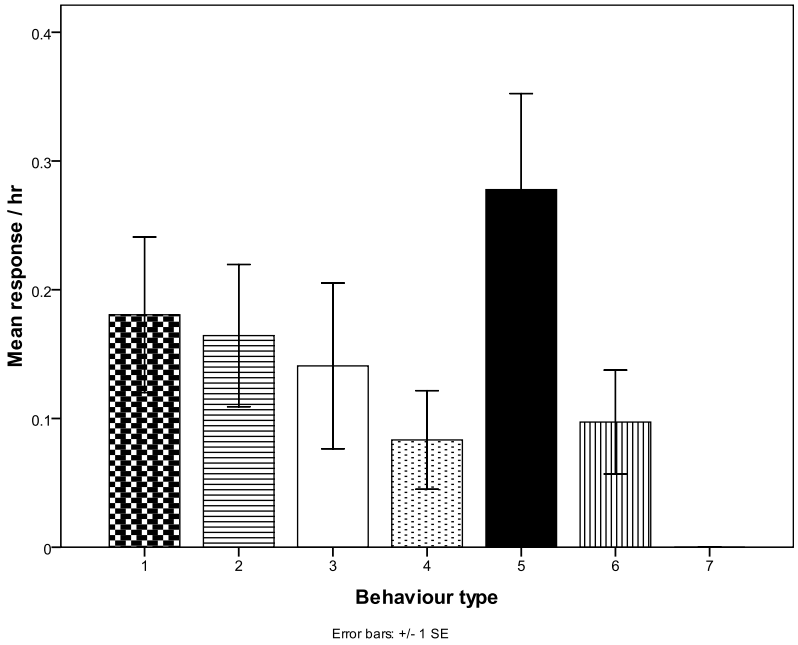


Figure 6.5: The mean response rate / hr of the 24 radio-tagged females to each behavioural category. There was a significant difference in the mean response rate per hour between categories (Kruskal-Wallis ANOVA H = 17.818, df = 6, p = 0.0007). However, when category 7 (unclassified behaviours) was excluded no significant difference was found in the mean response rate per hour between categories (Kruskal-Wallis ANOVA H = 7.378, df = 5, p = 0.194).

Source of variation	**SS**	**SS/MS total**	**d.f.**	***P* - value**
Month	2.629	2.308	4	0.6796
Behaviour response type	0.598	10.151	5	0.0721
Month*Behaviour response type interaction	3.879	14.977	20	0.7782

Table 6.6: The results of the Scheirer–Ray–Hare test showed that there were no significant differences in relation to month, behavioural response type nor a significant interaction between month and behavioural response type.

Source of variation	**SS**	**SS/MS total**	**d.f.**	***P* - value**
Range location	4.593	17.73	2	0.0001
Behaviour response type	1.768	6.82	5	0.2342
Range location *Behaviour response type interaction	2.014	7.77	10	0.6513

Table 6.7: The results of the Scheirer–Ray–Hare test showed that there were significant differences in the number of responses in relation to range location, but no significant differences in behavioural response type nor evidence of an interaction between range location and behavioural response type.

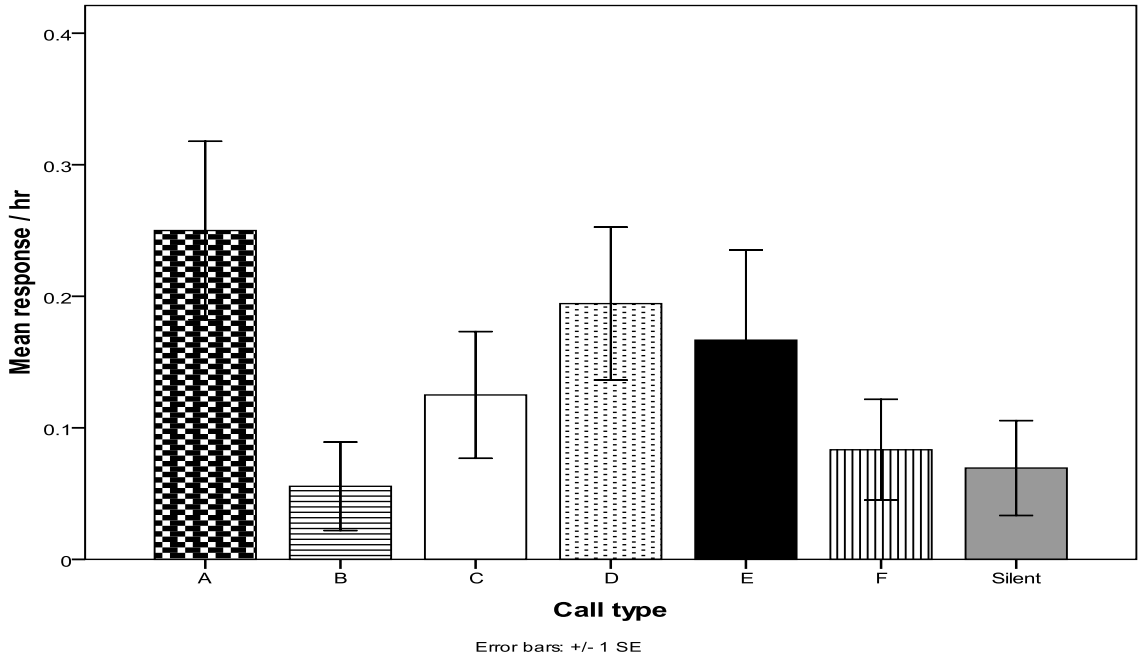


Figure 6.6: Mean number of responses per hour, of radio-tagged females, to each call type (Kruskal-Wallis ANOVA H = 17.818, df = 6, p = 0.022).

Call type	A	B	C	D	E	F	Silent
A		**Z = - 2.813, n = 144, p = 0.005**	Z = - 1.627, n = 144, p = 0.454	Z = - 0.490, n = 144, p = 0.624	Z = - 1.362, n = 144, p = 0.173	Z = - 2.211, n = 144, p = 0.027	Z = - 2.504, n = 144, p = 0.012
B			Z = - 1.301, n = 144, p = 0.193	Z = - 2.415, n = 144, p = 0.016	Z = - 1.558, n = 144, p = 0.119	Z = - 0.715, n = 144, p = 0.475	Z = - 0.381, n = 144, p = 0.703
C				Z = - 1.174, n = 144, p = 0.240	Z = - 0.272, n = 144, p = 0.786	Z = - 0.613, n = 144, p = 0.540	Z = - 0.942, n = 144, p = 0.346
D					Z = - 0.906, n = 144, p = 0.365	Z = - 1.779, n = 144, p = 0.075	Z = - 2.087, n = 144, p = 0.037
E						Z = - 0.881, n = 144, p = 0.378	Z = - 1.205, n = 144, p = 0.228
F							Z = - 0.338, n = 144, p = 0.735

Table 6.8: Pairwise comparison between different response rates for each call type. Using a critical value of 0.007, the only significant difference in response rates for radio-tagged females to each call type was between call type A and B, where call A elicited significantly more responses than call B.

Source of variation	SS	SS/MS total	d.f.	*P* - value
Month	0.570	2.436	4	0.6796
Call type	2.531	10.816	5	0.0552
Month*Call type interaction	4.299	18.372	20	0.5629

Table 6.9: The results of the Scheirer –Ray – Hare test showed that there was no significant differences in relation to month call type or evidence of a significant interaction between month and call type for radio-tagged females.

Source of variation	SS	SS/MS total	d.f.	*P* - value
Range location	3.868	16.53	4	0.6796
Call type	1.892	8.080	5	0.0552
Range location*Call type interaction	1.927	8.234	20	0.5629

Table 6.10: The results of the Scheirer –Ray – Hare test showed that there was a significant difference in the mean response rate per hour in relation to range location but no significance difference in relation to call type or evidence of a significant interaction between call type and range location.

Behaviour Response category	J-T – Statistic	df	*P*_value
1	0.978	5	0.3281
2	0.725	5	0.4692
3	1.136	5	0.2854
4	1.521	5	0.1281
5	1.652	5	0.0962
6	1.337	5	0.1813

Table 6.11: The results of the Jonckeere's trend test indicated that the radio-tagged female responses did not follow a predicted pattern in relation to season (using the non-tagged bats responses as the specified order of response).

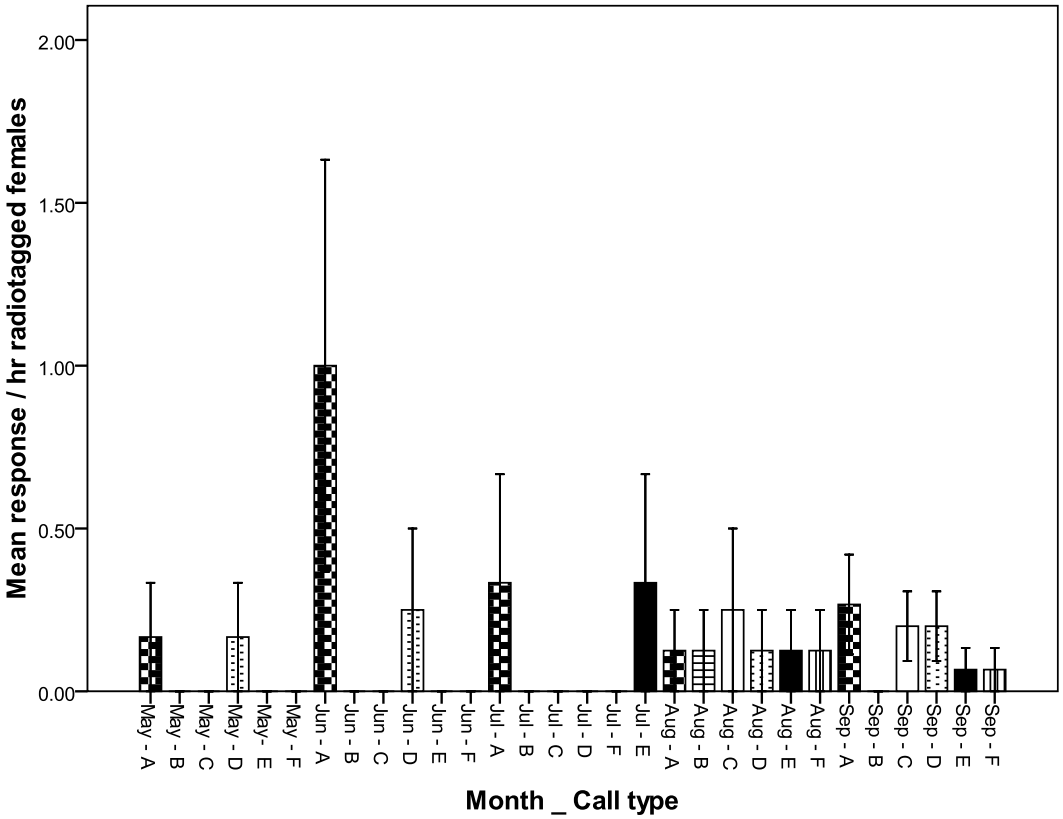


Figure 6.7: Mean response rate per hour for each call type in each month for radio-tagged females.

Call Type	J-T – Statistic	df	*P*_value
A	1.395	5	0.1632
B	0.425	5	0.6708
C	3.126	5	0.0018
D	0.971	5	0.3315
E	0.232	5	0.8165
F	0.545	5	0.5858

Table 6.12: The results of the Jonckeere's trend test indicated that the radio-tagged female responses followed a predicted pattern in relation to season (using the non-tagged bats responses as the specified order of response) for call C only.

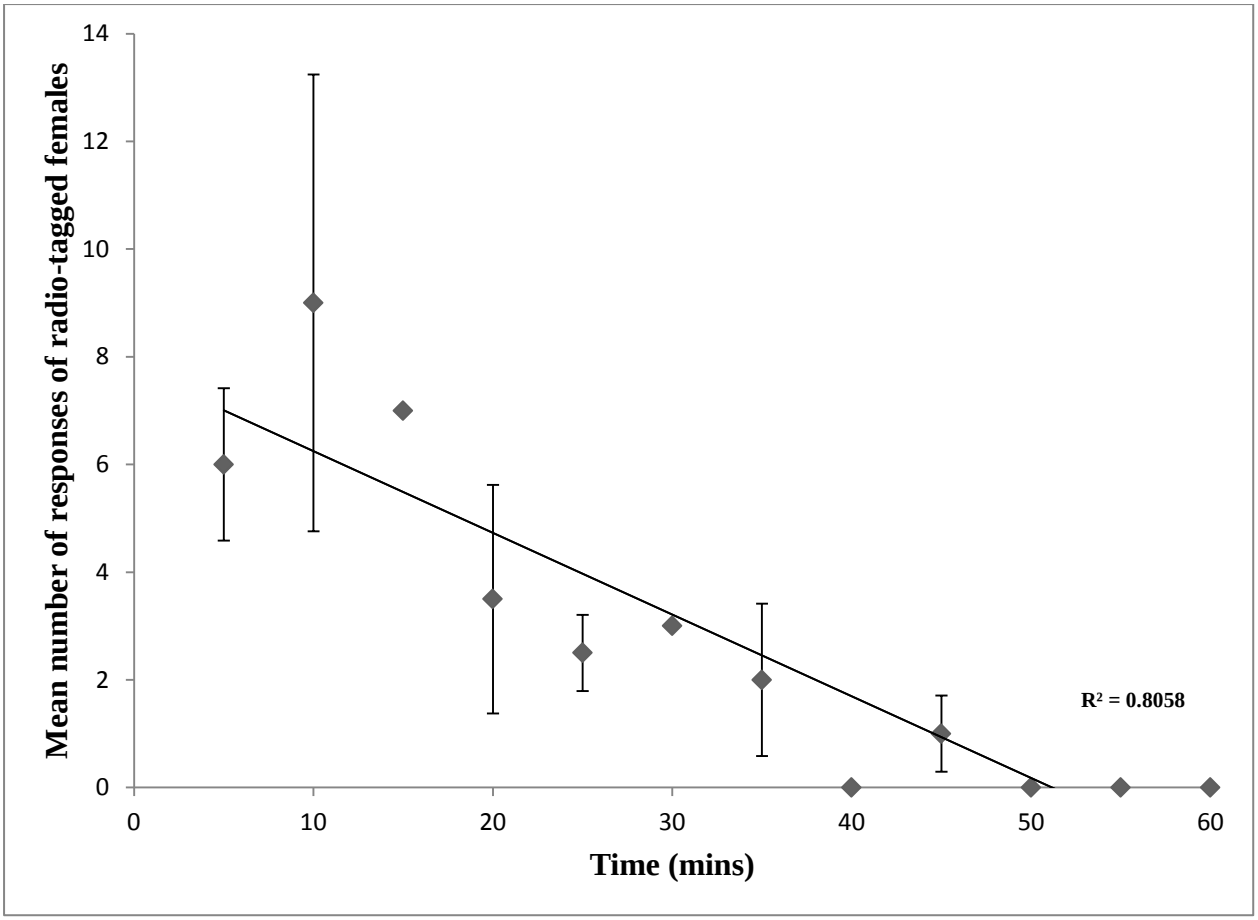


Figure 6.8: Mean number of responses to synthesised social calls broadcast over a 60 minute experimental period. The error bars show the standard deviation between years (i.e. means in 2007 and 2008). The mean number of responses was negatively correlated with experimental play time, i.e. most radio-tagged females responded within the first 15 – 20 minutes of the stimulus broadcast).

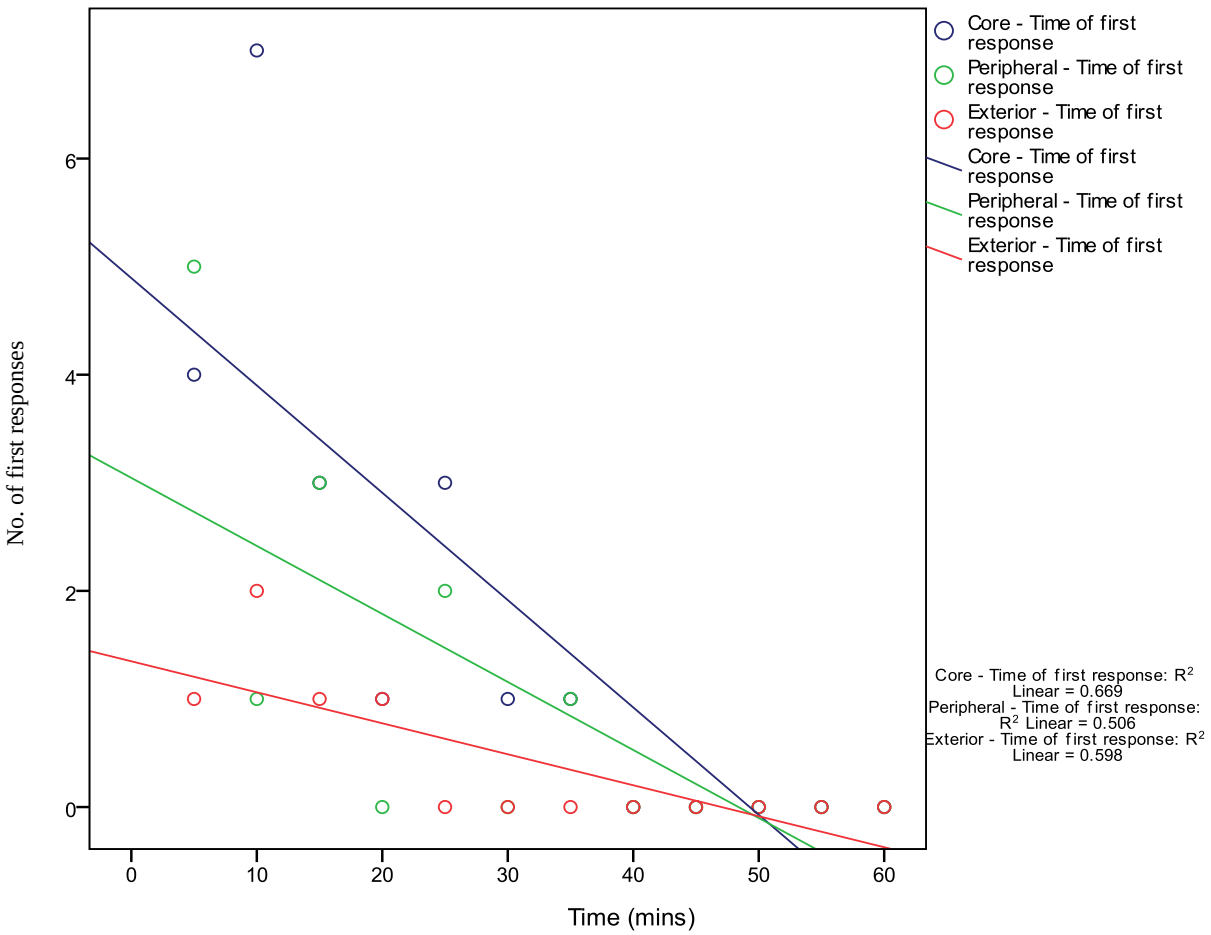


Figure 6.9: The number of first responses to simulated *P. auritus* social calls by radio-tagged *P.auritus* in relation to their core, peripheral and exterior foraging localities. Most responses occurred within the first 30 minutes and the time of first response was negatively correlated with time at all localities.

7.1 - Introduction

There was evidence of spatial overlap in home range use between female *P. auritus* radio-tracked at a variety of sites (Chapter 5). The amount of overlap in individual home ranges can provide indirect information about the likelihood of social interactions (Shier and Randall, 2004; Chaverri et al., 2007; Harless et al., 2009) and has been described as an index of 'static interaction'(Macdonald et al., 1980). However, such an index is of limited value because it does not take into account the utilisation distribution of shared parts of the range (Doncaster, 1990). For example, two females may have an area of overlap in their foraging ranges but seldom meet there because most of their foraging activities are concentrated in non-overlapped areas, or they use the overlapping areas at different times. On the other hand, two females with a similar area of overlap may concentrate their foraging activities on that common area.

Simultaneous radio-tracking of two or more individuals allows each animal's movements to be analysed in relation to the movements of other individuals. This analysis, termed 'dynamic interaction', uses the simultaneous locations to assess temporal aspects of interaction within the context of home range use (Doncaster, 1990; Minta, 1992). Dynamic interaction analysis has been used in a variety of radio-tracking studies to investigate the interactions of animals such as badgers (Bohm et al., 2008), foxes (White and Harris, 1994), and coyotes (Chamberlain et al., 2000). In these studies, interactions are defined as two individuals being recorded within a critical distance of one another which, in turn, is determined by the radio-tracking regime.

The overlap of home range means that there is the potential for females to coordinate their foraging activities. Co-ordinating foraging through passive information transfer has been proposed as a reason for group formation of male bats in species foraging for ephemeral insects in temperate zones (Safi and Kerth, 2007). More active information transfer has been demonstrated by Wilkinson and Boughman (1998) in greater spear-nosed bats *Phyllostomus hastatus*. In this species screech calls appear to act as contact calls that recruit other members of the colony to rich food resources, thereby coordinating foraging among group members. If *P. auritus* females overlap considerably in home range, and show some evidence of co-ordinating foraging, then the responses to the Autobat stimulus calls could represent attraction to what they perceive as recruiting calls, in which case the resource defence hypothesis may not account for female propensity to respond.

This study aimed to: (i) estimate the foraging range overlap of simultaneously radio-tracked dyads caught at the same location; (ii) assess the extent to which roosting associations and overlap of foraging areas are related (iiii) examine the interactions between simultaneously radio-tracked *P. auritus* females in relation to their movements; (iv) determine the extent of range and core area overlap of non-simultaneously radio-tracked females radio-tracked at the same site within the same year. If responses to the Autobat stimulus represent approaches to what are perceived as recruitment calls, then it would be expected for female ranges to overlap considerably and show some evidence of co-ordination of foraging between pairs of females that share their range.

7.2 - Methods

7.2.1 - Capture and selection of study animals

Between August 2007 and September 2008 20 females, comprising 10 pairs, were captured at the study sites and fitted with radio-transmitters, as detailed in Chapter 5. Bats were caught in pairs, either in mist nests or harp traps, to which they were attracted using an acoustic lure (Sussex Autobat: Hill & Greenaway, 2005) that produced synthesised social calls (as detailed in Chapter 4). The pairs of females were always caught and radio-tagged on the same night. In order to maximise the chances of catching a pair with adjacent or overlapping foraging areas two capture sites (occasionally three) were located in the woodland interior away from edges and rides at a distance of approximately 50 metres apart. Trapping began approximately 60 minutes after sunset and lasted for approximately 2 hours. If more than two females were caught at the two capture sites, individual females caught in the same trap or net were selected for radio-tagging in preference to females caught in two traps or nets. If more than two females were caught in the same trap then those females caught closer together in time were selected for radio-tagging.

7.2.2 - Radio-tracking methodology and home range analysis

For each pair of females the day roosting location of each individual was recorded over the radio-tracking period and the females were radio-tracked synchronously from emergence to re-entry. Synchronous radio-tracking involved taking bearings from one individual from the dyad followed immediately by taking bearings from the other member of the dyad. Simultaneous radio-tracking of dyads allows for the calculation of separation distances at five minute intervals in order to assess direct interactions and movement patterns with respect to

each other. If one member of the dyad moved too far away from the other to be simultaneously radio-tracked then, if possible, the surveyors would switch between individuals at 15 minute intervals in order for accurate bearings to be taken. Pairs of compass bearings, and the locations they were taken from, were used to estimate the locations of the foraging *P.auritus* dyads. Home range boundaries were defined by 100% MCP and 95% KDE and the core foraging areas were determined by 50% KDE (as detailed in Chapters 5 and 6).

7.2.3 - Overlap analysis

The degree of overlap of simultaneously radio-tracked dyads and, also, of individuals that were radio-tracked, at the same site in the same year (but not simultaneously), was assessed by calculating the proportional overlap of all individual foraging ranges 100% MCP, 95% KDE, 50% KDE using the program Ranges7eXtra v1.8 Anatrack Ltd. Percentage range overlap of each home range estimator was calculated in Ranges using the formula:

$$(Rzy/Rz + Ry) \times 2$$

where *Rzy* represents the size of the region of overlap between bats *z* and *y*, and *Rz* and *Ry* represent the total range size of bats *z* and *y*. By multiplying this number by 2, the average area of overlap within each bat's range was obtained.

An index of range overlap was also calculated for simultaneously radio-tracked individuals. An association index (used also by Chamberlain et al., 2000 which modified the simple ratio used by Ginsberg and Young (1992) to quantify association:

$$n_1 + n_2 / (N_1 + N_2) \times 100$$

where n_1 and n_2 are the number of locations for the bats within the overlap region and N_1 and N_2 are the total number of fixes recorded for each bat. An index of roosting association for each dyad was also calculated by dividing the number of days the females shared roosts by the total number of days their roosting location was recorded. Therefore, if the simultaneously radio-tracked dyad shared the same roost every day the index would give a value of 1. On the other hand, if they did not share a roost during the radio-tracking period, the index would give a value of 0. This index of roosting association was used to investigate whether the degree of foraging range overlap was related to how frequently pairs of females roosted together or not.

7.2.4 - Spatial Association

A grid-based spatial analysis of the estimates of locations for each dyad was applied to test for association using a pair-wise comparison test of association (Schluter, 1984). Spatial Association is a grid-based test that compares the presence or absence of different point patterns in each grid quadrat. Pairwise results are based on a Chi-squared test between all possible pairs of point patterns selected for comparisons. Yates correction factor was applied to account for bias resulting from cases of low cell frequencies, reducing the chi-square value obtained and, thus, increasing its p-value (Armitage and Berry, 1994).

The Jaccard index (also known as the Jaccard similarity coefficient) is a statistic used for comparing the similarity and diversity of sample sets. The Jaccard similarity index considers the similarity between two units as the number of attributes shared divided by the total number of attributes present in either of them (Waite, 2000) and may be expressed as follows:

Jaccard Index: $J(A,B) = |A \cap B| / |A \cup B|$.

The Jaccard similarity index was calculated for each dyad using estimates of location in Biotas 1.03, Ecological Software Solutions Ltd.

7.2.5 - Dynamic interaction analysis

Overlap in range area does not necessarily indicate that the individuals frequently encounter each other because they may rarely visit the same place at the same time (Macdonald et al., 1980). Doncaster (1990) called the analysis of overlapping range 'static interaction' (described above) and proposed the examination of 'dynamic interaction' by looking at estimates of locations taken at the same time. Analysis of dynamic interactions, as described by Doncaster (1990), compares the *n* observed separation distances from simultaneously radio-tracked pairs against baseline data made up of the n^2 artificially paired separation distances from the same sample using χ^2 tests. However, it has been questioned whether the comparison of 'real' separation distances with unpaired separation distances, which bear no resemblance to a biologically meaningful movement path, undermines the validity of the test (White and Harris, 1994). Furthermore, the original publication, which provided a test of whether two individuals showed significant attraction or avoidance, depended on two assumptions: firstly, that locations are statistically independent and secondly, that their distribution fitted a parametric model. It was not possible to assume independence from the distribution of locations between single pairs of females.

To investigate whether paired females were more cohesive than expected by chance, a single statistic for each range was compared using observed and possible distances between animals, as recommended by Kenward et al., (1993). The mean, geometric mean and median distances were estimated between the n observed pairs of same-time locations for animals one and two. The equivalent values were then estimated for the n x n possible distances (if animal two could be at any of its n used positions when animal one was at each of its used positions). The observed and possible distances were subsequently compared using the Jacobs Index (Jacobs, 1974), which gives a value of 0 if the observed and possible distances were the same, rising towards +1 if observed distances were small relative to possible distances (because the animals were usually together) or falling towards -1 if animals tended to avoid each other. The Jacobs index formula is:

$$D = \frac{de - do}{de + do}$$

(where *de* is the expected distance and *do* is the observed distance between pairs of simultaneous locations). This gives a single index for each pair of animals, which tends to be most consistent if based on the geometric mean distances (Walls and Kenward, 2001). The existence of a dynamic interaction does not necessarily imply mutual awareness on the part of the respective animals (Chamberlain et al., 2000), but addresses whether animals are more likely (positive interaction) or less likely (negative interaction) to maintain a certain separation distance than would be expected from the configuration and utilisation of areas within their known home ranges (Doncaster, 1990).

7.3 - Results

7.3.1 - Overlap of simultaneously radio-tracked females

Thirty-six nights of simultaneous radio-tracking were recorded from 10 different dyads. One dyad showed no overlap in any measure of range area. Another dyad showed less than 3% overlap in the 100% MCP, and no overlap in other measures (Pairs three and five, detailed in Appendix A.7.1). The percentage overlap for the remaining eight dyads (16 individuals) varied and ranged from 23.4% to 92.3% (mean 51%; sem = 4.9%) in the 100% MCP; 17.3% to 97.9% (mean 53.5%; sem = 5.8%) in the 95% KDE; and 17.5% to 96.8% (mean 46.8%; sem 6%) in the 50% KDE. There was no significant difference between the mean amounts of overlap, for each female, between the three range estimators (100% MCP, 95% KDE and 50% KDE) (Kruskal-Wallis ANOVA $H = 1.474$, $n = 48$, $p = 0.479$). This variation in the extent of overlap is exemplified by dyads radio-tracked at Capite Wood. Pair three (Bleb 17 and 18), were caught at the same location in the woodland and subsequent radio-tracking data revealed that this pair had no overlap in any of the range estimators employed. However, by comparison, pair eight (Bleb 27 and 28), also caught at the same location had approximately 25% overlap in the 100% MCP and 95% KDE and approximately 20% in the 50% KDE for each individual, as illustrated in Figures 7.1, 7.2 and 7.3.

7.3.2 - Spatial Association

The grid-based spatial association test (Schluter, 1984) was used to compare the presence or absence of different point patterns (estimates of location) in each grid across the range. The

test found that there was a significant positive spatial association for eight of 10 dyads, as detailed in Table 7.1. Two dyads (pair three and pair five) had a negative association. For example, at Capite Wood pair three had no association (Figure 7.4a) as there were no grids that shared estimates of location. Whereas, by comparison, pair eight had a positive association (Figure 7.4b), as they were nine grids sharing estimates of location from both females.

7.3.4 - Range overlap and roosting association of simultaneously radio-tracked P. auritus

For each dyad both females were located in their roosts on between three and six days (Table 7.2). For three pairs the females were always found roosting separately (roosting index = 0), while for three pairs the females were always found in the same roost (roosting index=1). The remaining four pairs were found roosting together on some days and apart on others. There was a significant positive correlation between roosting index and the Jaccard index (Spearman $r^2 = 0.876$, $p<0.0009$), indicating that dyads with greater overlap in their ranges roosted together more often (Figure. 7.5).

7.3.5 - Dynamic interactions of simultaneously radio-tracked dyads

A total of 1044 ‘paired observations’ were obtained from the ten dyads throughout the radio-tracking period. The number of paired observations for each dyad ranged from 37 to 188 (mean 104.4; SD = 50.05). The mean distance between pairs of fixes for each dyad ranged from 50.70 metres to 251.88 metres (mean 131.05; SD = 68.38). The Jacob index value for each dyad ranged from 0.006 – 0.315 (mean = 0.142; SD = 0.130). Table 7.3 shows the results of the dynamic interaction analysis. Six of the ten adult female pairings were close to

zero, indicating neither avoidance nor attraction during foraging. One pair, pair six, lactating females who shared the same roost, did show evidence of a positive interaction (Jacob index = 0.315). Adult – juvenile pairings (n = 3) had a tendency to have a greater interaction when foraging, (Jacob index, 0.230, 0.292, 0.312 respectively), suggesting that adults and juveniles may associate more frequently compared to adult females when foraging (Fishers Exact Probability 2 x 3 p = 0.0333). The distance between paired observations for each dyad was categorised into distance bands of 50 metre intervals. The percentage of observations in each distance category, for each dyad, is shown in Table 7.4.

7.3.6 - Range overlap of all simultaneously and non-simultaneously radio-tracked individuals radio-tracked at the same site within the same year

Overlap was also found in the foraging ranges of females that were not radio-tracked simultaneously (but were radio-tracked within the same year). The proportion of overlap, in the 100% MCP, 95% KDE and 50% KDE, for each female with other females radio-tracked at the same site, in the same year, are detailed in the Appendix (A.7.2, A.7.3 and A.7.4). The amount of overlap did vary between sites. However, a comparison is not possible due to the likelihood that only a small proportion of the bats from each site were radio-tracked. Nonetheless, the overlap between adjacent females at one site, Hoe Wood, was extensive. For example, one female (Bleb 24) range (95% KDE) overlapped by 50% with four other females. Her core area also overlapped extensively (27% to 52%) with those four females. Figure 7.6 shows the overlap in the core foraging area of six females radio-tracked at Hoe the 14th July 2008 and the 11th August and 2008. Overlap in the core area for females at this

woodland was common, indicating that females were unlikely to attain range or core foraging area exclusivity.

7.4 - Discussion

Territoriality has been defined in various ways, a common one being the defence of a fixed spatial area to exclude other animals (Noble, 1939; Kaufmann, 1983; Maher and Lott, 1995). Territoriality can also be defined as the exclusive use of fixed space (Krebs, 1970; Kerr and Bull, 2006) or as a range which an animal has site-specific dominance (Emlen, 1957) and priority of access to resources over others, is achieved through social interactions such as advertisement behaviour and contests (Kaufmann, 1983). This 'exclusive use of fixed space' may refer to individuals, pairs or groups of animals defending an area and the degree of exclusive use varies enormously across taxa. How animals utilise space has important consequences for social organisation, foraging behaviour and mating. Territorial behaviour, in which a territory is defended by means of aggressive displays and/or threats, has been demonstrated empirically for many species of birds and fish (for review see Maher and Lott 2000). It has also been reported for some species of rodents (Beletsky and Orians, 1989) and primates (da Cunha and Byrne, 2006).

Studying territorial behaviour in bats is problematic because they are fast flying and nocturnally active so the likelihood of a researcher observing social interactions among conspecifics is low. One alternative for elusive mammals is to study the overlap in range use. Therefore, the amount of home range overlap or, conversely, range exclusivity is generally used as an index to infer territoriality in which territoriality is equated with 'little' overlap and absence of territoriality with 'more' overlap, with no set criterion of when a home range

becomes a territory (Maher and Lott, 1995). Bradbury (1977) concluded that female territoriality is virtually absent among bats, given the ephemeral nature of the distribution of prey items (Bradbury, 1977). However, Rydell (1986) described a scenario in which reproducing female northern bats *Eptesicus nilssoni* defended feeding territories against other colony members as well as non-members by means of aggressive chases and vocalisations (Rydell, 1986).

In this study, home range overlap was used as an index to infer the spacing patterns of females. The results of the current study show that the home range of *P.auritus* females may overlap considerably with one or more conspecific females. Overlap in female home ranges has been reported for various animals including coyotes *Canis latrans* (Chamberlain et al., 2000), black-footed ferrets *Mustela nigripes* (Jachowski et al., 2010), and eastern grey kangaroos, *Macropus giganteus* (Carter et al., 2009). However, other animals, such as European moles *Talpa europaea* (Macdonald et al., 1997), desert tortoises (Harless et al., 2009), and short-tailed mongoose *Herpestes brachyurus* (Jennings et al., 2010), do not exhibit such sharing tendencies amongst females, but often have range exclusivity.

Studies investigating spacing patterns in bats by means of radio-tracking have reported a large amount of variation in both range size and the extent of overlap between individuals. Table 7.5 summarises the findings of various radio-tracking studies on bats which have reported the home range overlap. The size of home range does not appear to be a good predictor for the extent of home range overlap, as species exhibiting large home ranges, such as the Barbastelle bat *Barbastella barbastellus* (Hillen et al., 2009), had low levels of overlap for radio-tracked females, as did Bechsteins bat *Myotis bechsteinii* (Kerth et al., 2001) whose

home ranges were small (Table 7.5). However, when making these comparisons, it must be acknowledged that, many of the studies, including this one, have only radio-tracked a small proportion of the bats. Therefore, the picture of the degree of overlap is likely to be incomplete.

Indirect evidence for overlap in the foraging areas of *P. auritus* has also been reported by Swift and Racey (1983), in which observations of bats marked with reflective tape recorded up to 'five *P. auritus* feeding together without any obvious interaction' (Swift and Racey, 1983). Entwistle (1994) simultaneously radio-tracked one pair of female *P. auritus* and found that they spent 11% of their time within the same feeding site. In addition, previous radio-tracking work indicated that there may be overlap in the feeding ranges of *P. auritus* (although the sample size was too small to be conclusive) (Murphy *et al.* unpublished data). Furthermore, as detailed in Chapter 4, 23% of filmed responses involved multiple individuals responding concurrently. This indicates that the individuals responding were likely to be in reasonable close proximity to one another when they were motivated to respond to the stimulus.

Some studies have reported animals overlapping in home range use but having exclusive core areas. For example, the dusky-footed wood rat *Neotoma fuscipes*, a nocturnal mammal, showed home range overlap with multiple neighbouring woodrats, both same sex and opposite sex, suggesting that foraging areas were shared. However, there was little overlap between same – sex neighbours in core foraging areas (Innes et al., 2009). Core area exclusivity has also been demonstrated for many animals including sleepy lizards *Tiliqua rugosa* (Kerr and Bull, 2006), wild ferrets *Mustela furo* (Norbury et al., 1998), and yellow-necked mice (Stradiotto et al., 2009). The simultaneously radio-tracked female *P. auritus*

clearly did not have exclusive use of their core areas, as the mean overlap in core area between dyads was 37.4%. Furthermore, the data on the non-simultaneously tracked females (radio-tracked over a four week period) at Small Dole suggests that *P. auritus* females may also exhibit extensive overlap in their core foraging areas in addition to their overlap in their foraging range. Overlap in core foraging areas (23-56%) has also been reported in the New Zealand long-tailed bat *Chalinolobus tuberculatus* (O'Donnell, 2001; Greaves et al., 2006). However, these studies used core foraging area estimates of 85% clusters as their range distributions were highly skewed.

Strong colony fidelity has been reported in various studies for *P.auritus* (Heise and Schmidt, 1988; Benzal, 1991; Entwistle, 1994) and, where such coloniality is coupled with female recruitment into the natal group and subsequent long-term philiopatry, colonies will be comprised of female kin originating from one or more matrilines e.g. (Wilkinson, 1985; Kerth et al., 2000; Rossiter et al., 2002). High relatedness amongst colony members may favour the evolution of cooperative behaviour, such as sharing resources within foraging areas, via kin selection (Hamilton, 1964). In this study, the relatedness levels of *P. auritus* were not assessed but a significant positive correlation between roosting association and foraging range overlap was found. All but one pair of dyads whose ranges overlapped roosted together at least once during the radio-tracking period, and the seven pairs that shared a roost during the brief radio-tracking period are, therefore, likely to have been from the same colony. Two of the three pairs that did not share a roost during the brief radio-tracking period also showed no overlap. It is possible that these two pairs were not from the same colony. A study on range overlap and roosting association in the tent-making bat *Artibeus watsoni* also found a significant positive correlation between range overlap and roosting association (Chaverri et al., 2007). Studies specifically examining relatedness as an explanation for the

extent of home range overlap have also found that the degree of overlap between individual foraging areas was significantly positively correlated with degree of genetic similarity (Kerth et al., 2001; Rossiter et al., 2002)

Radio-tracking studies that have involved simultaneously (or near simultaneously) recorded locations of two or more individuals have defined interactions occurring when two or more individuals are found within a critical distance of each other (determined by the radio-tracking regime). Such studies, for example on coyotes (Chamberlain et al., 2000), have found evidence of significant interactions whereby the authors interpret adult female coyotes 'positive interactions' as an indication that they were travelling together more often than expected by chance. A study on the interactions of grizzly bears *Ursus arctos* found that female-female interactions were more likely to occur at a greater frequency during a particular time of year (i.e. the berry season) (Stenhouse et al., 2005). By contrast, a study on the encounters of red foxes in Bristol found that, for most of the year, foxes practised avoidance and incursions into neighbouring territories were rare and, thus, conflicts were minimised, presumably because of the high risks involved with aggressive encounters (White and Harris, 1994). However, studies on the dynamic interaction of wild female ferrets *Mustela furo* (Norbury et al., 1998) and European moles *Talpa europaea* (Macdonald et al., 1997) have found no evidence of attraction or avoidance of simultaneously tracked dyads.

The dynamic interaction analysis, found no overall evidence of either attraction or avoidance for the simultaneously radio-tracked dyads. However, females radio-tracked in synchrony with juveniles did show evidence of cohesion. If these were mother-daughter pairs then this association would be expected, as studies of several species have found that females and their newly volant offspring frequently forage together (the yellow winged bat *Lavia frons*

(Vaughan and Vaughan, 1987), the big-brown bat *Eptesicus fuscus* (Brigham and Brigham, 1989) and the greater horseshoe bat *Rhinolophus ferrumequinum* (Rossiter et al., 2002)). Alternatively, it could be that juvenile *P. auritus* tend to associate more with adult individuals within their colony as juvenile northern long-eared bats *Myotis septentrionalis*, for example, have more direct and indirect associations with adult females, which may be a consequence of juveniles tending to be more exploratory (Patriquin et al., 2010).

The absence of an interaction between the female dyads that shared foraging sites may imply that the sharing of space, and resources within that space, is a common mechanism for female colony members. This study has demonstrated that although simultaneously radio-tracked pairs of females were spatially associated with one another, they were neither attracted to nor avoided one another when foraging. It is possible, however, that females occasionally came together attracted by calls to share particularly rich food sources. It could be that a female forages alone for the majority of the time but shows co-operative foraging tendencies by calling to advertise a rich food source, which in turn, attracts a conspecifc which heard her call and was motivated to respond. If this happened infrequently it may not be detected using radio-tracking methodology alone. However, an alternative explanation could be that females from the same colony are aware of one another's presence within their home range and can discriminate colony members from intruders, which may explain their propensity to respond to the Autobat stimulus.

Data on the distances between pairs of simultaneously radio-tracked females showed that 20% of the paired observations were within 50 metres of each other (a distance determined in Chapter 4 from where a *P. auritus* would reliably respond to the Autobat stimulus) and the majority of paired observations (approximately 78%) fall within the first five distance

categories (i.e. within 250 metres of the other female). The broadcasting of *P. auritus* simulated social calls within these foraging areas, and the resulting rapid response of one or more females, suggests that female *P. auritus* may co-operatively defend their shared resources against unknown intruders.

7.5 - Summary

- Female *Plecotus auritus* regularly shared foraging areas with conspecifics.
- For dyads simultaneously radio-tracked there was a significant correlation between roosting association and range overlap (i.e. dyads with greater overlap in their ranges roosted together more frequently).
- Although females regularly shared foraging areas, there was little evidence of co-ordination of movements between simultaneously radio-tracked dyads.

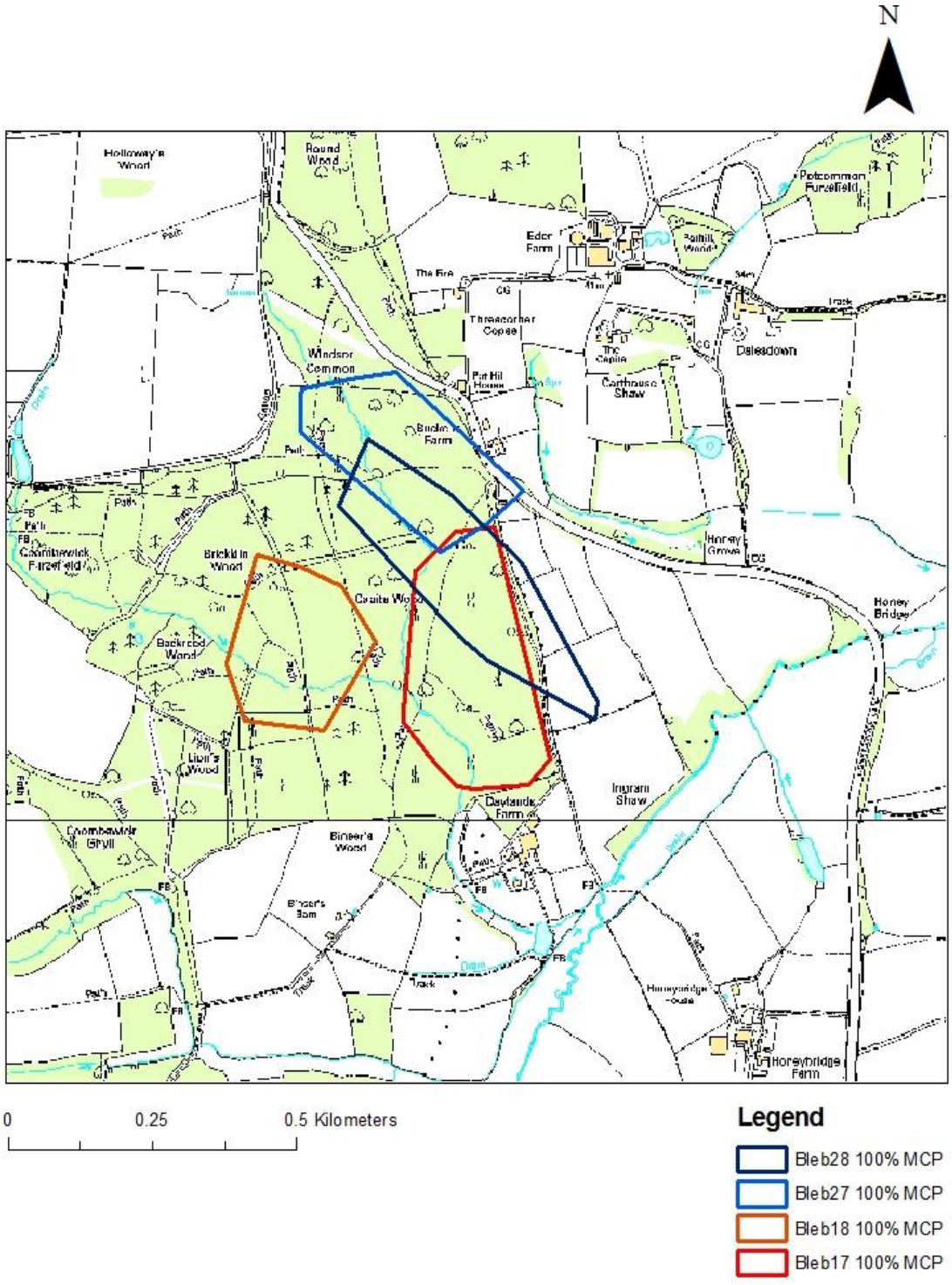


Figure 7.1: Range overlap of four female *Plecotus auritus* radio-tracked at Capite Wood, West Sussex in 2008. Bleb 17 (orange) and Bleb 18 (red) were caught at the same location and radio-tracked simultaneously. There was no overlap in the resulting 100% MCP for this pair of females. Bleb 27 (blue) and Bleb 28 (navy) were caught at the same location and radio-tracked simultaneously. There was overlap (29.13% of Bleb 27 100% MCP range and 24.32% of Bleb 28 100 % MCP range) for this pair of females and, also, overlap with Bleb 17 (not radio-tracked simultaneously).

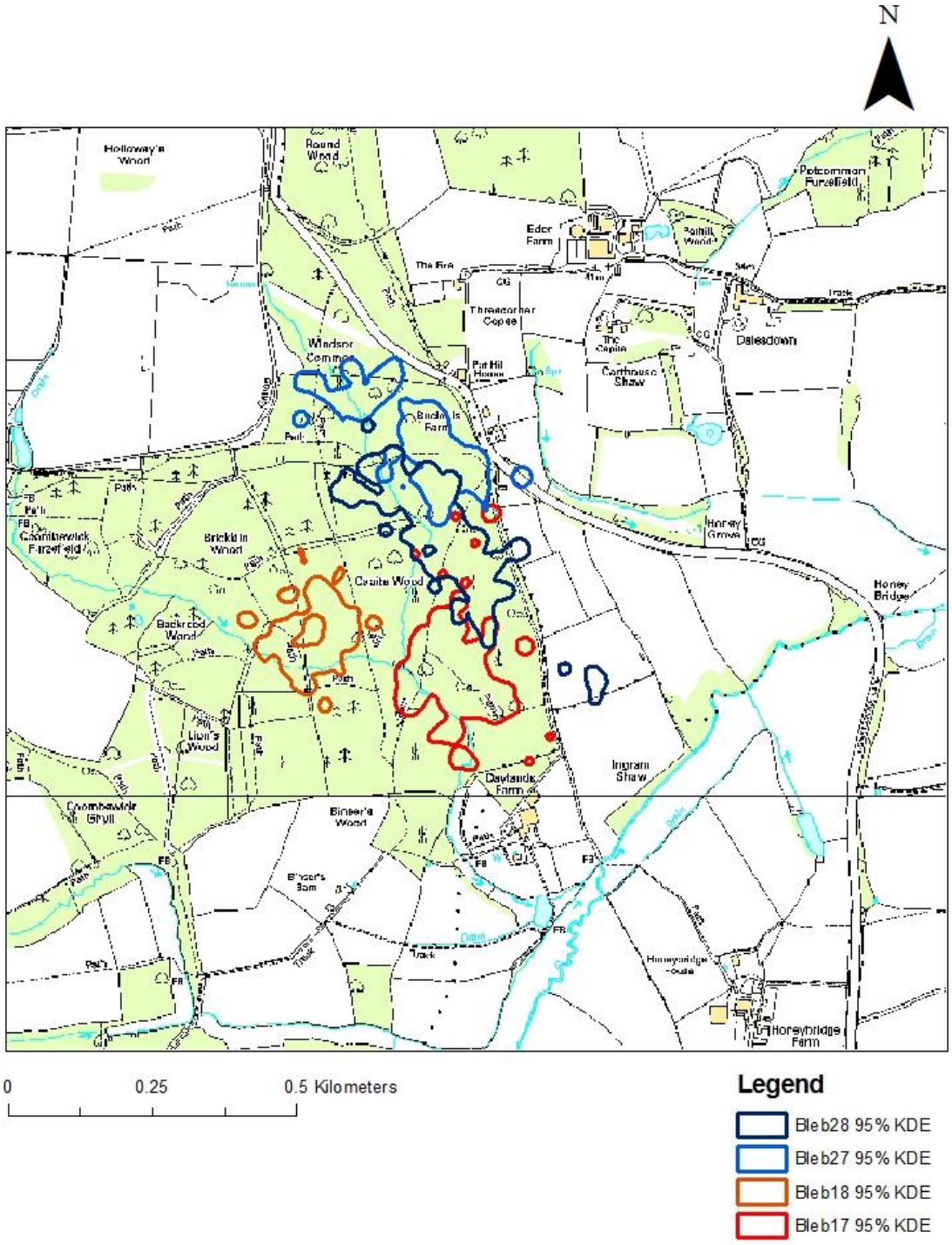


Figure 7.2: Range overlap of four female *Plecotus auritus* radio-tracked at Capite Wood, West Sussex in 2008. Bleb 17 (orange) and Bleb 18 (red) were caught at the same location and radio-tracked simultaneously. There was no overlap in the resulting 95% KDE for this pair of females. Bleb 27 (blue) and Bleb 28 (navy) were caught at the same location and radio-tracked simultaneously. There was overlap (24.05% of Bleb 27 95% KDE and 24.32% of Bleb 28 25.05 95 % KDE) for this pair of females. Bleb 28 also overlapped with Bleb 17 in the 95% KDE (not radio-tracked simultaneously).

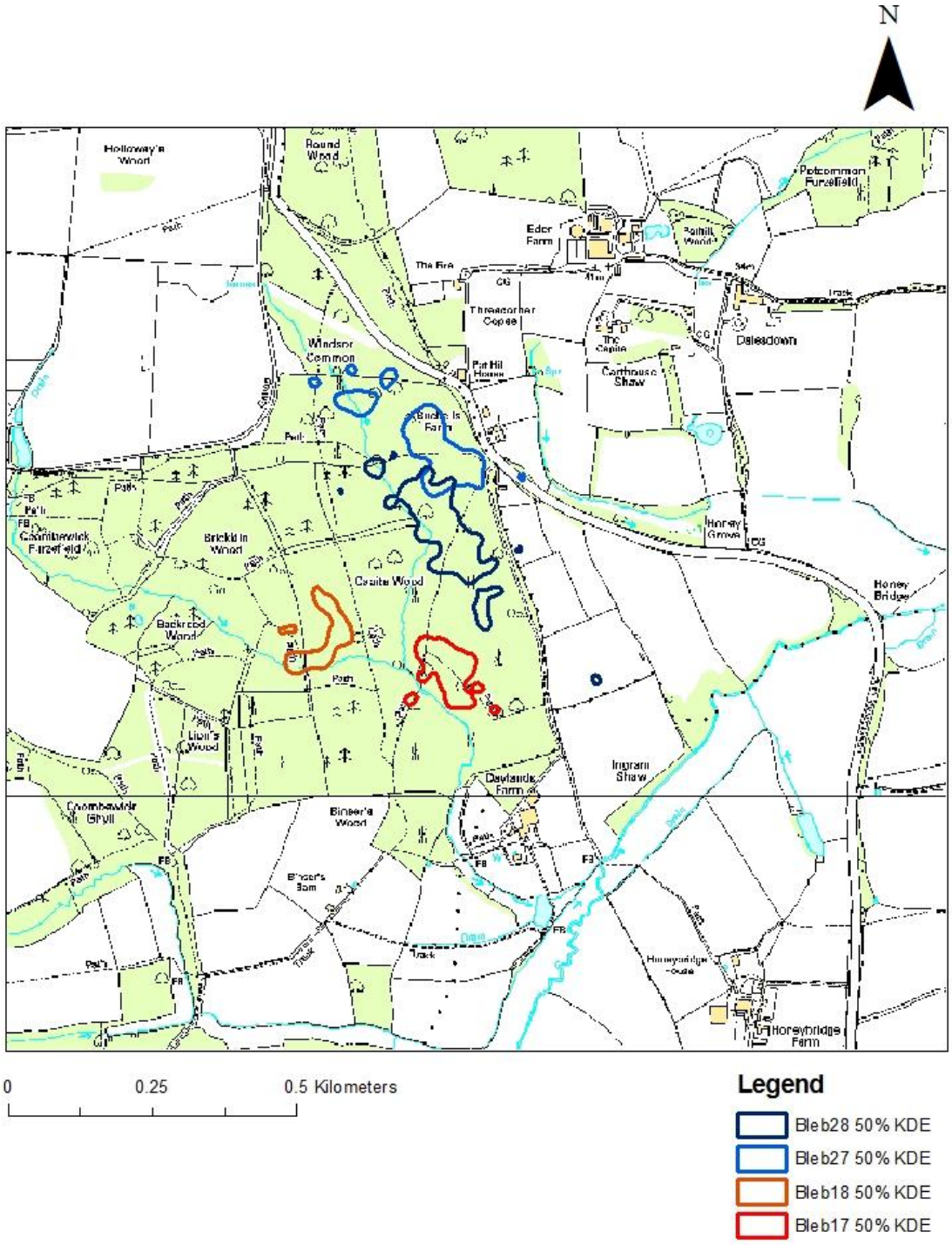


Figure 7.3: Range overlap of four female *Plecotus auritus* radio-tracked at Capite Wood, West Sussex in 2008. Bleb 17 (orange) and Bleb 18 (red) were caught at the same location and radio-tracked simultaneously. There was no overlap in the resulting 50% KDE for this pair of females. Bleb 27 (blue) and Bleb 28 (navy) were caught at the same location and radio-tracked simultaneously. There was overlap (17.54% of Bleb 27 95% KDE and 19.23% of Bleb 28 95 % KDE) for this pair of females.

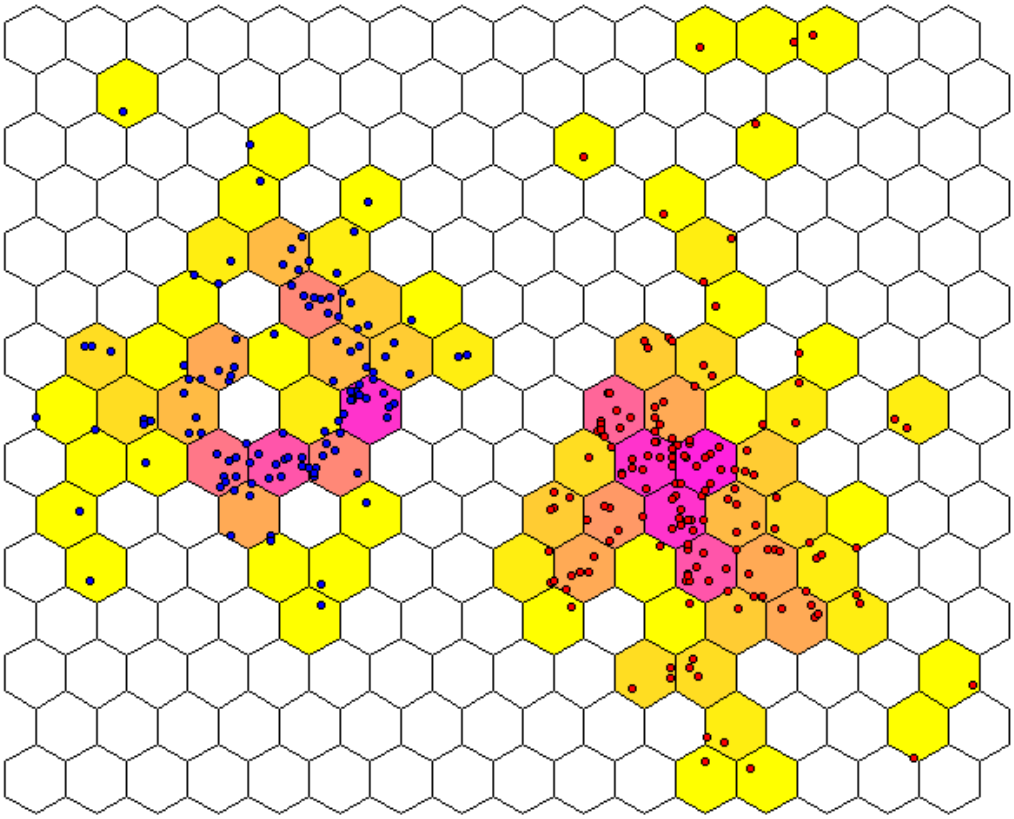


Figure 7.4 (a): Spatial association of the estimates of location (fixes) of two females (pair 3 – bleb 17 & 18), radio-tracked simultaneously at Capite Wood. The estimates of location for bleb 17 are shown by the red dots and the estimates of location for bleb 18 are shown by the blue dots. The Schluter test calculates a grid system over the estimates of location with the lighter colours (yellow) representing areas with 1 or 2 fixes (areas used less frequently) and the darker colours (pink) representing the clumping of fixes (or core areas). The number of grids where locations occur for both females, in this dyad is 0 and, therefore, the Schluter test has calculated a negative spatial association (X_2 7.821, n = 273, p > 0.005) and the corresponding Jaccard index is 0 (p < 0.001), as no grid squares had locations for both animals.

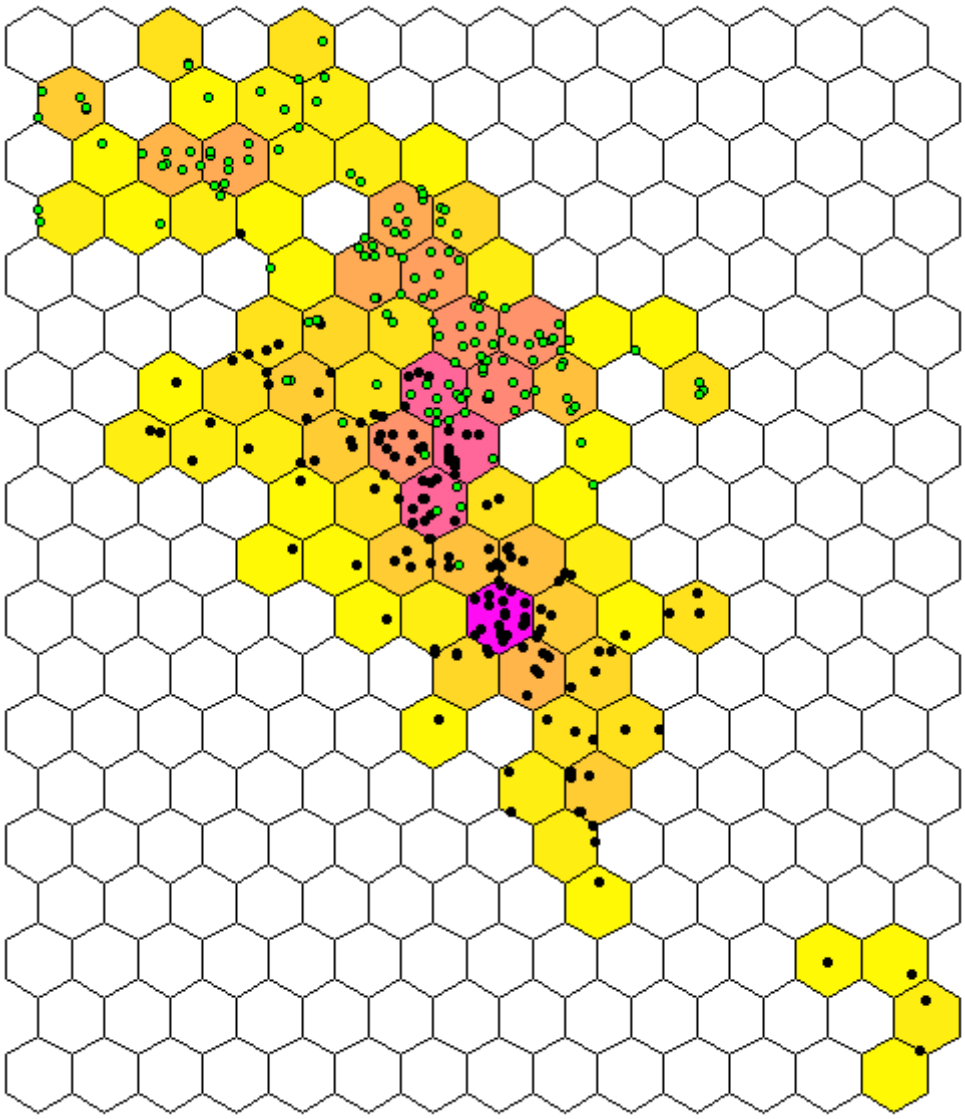


Figure 7.4 (b): Spatial association of the estimates of location (fixes) of two females (pair 8 – bleb 27 & 28), radio-tracked simultaneously at Capite Wood. The estimates of location for bleb 27 are shown by the green dots and the estimates of location for bleb 28 are shown by the black dots. The Schluter test calculates a grid system over the estimates of location with the lighter colours (yellows) representing areas with one or two fixes (areas used less frequently) and the darker colours (pinks) representing the clumping of fixes (or core areas). The number of grids where locations occur for both females, in this dyad is 9 and, therefore, the Schluter test has calculated a positive spatial association (X_2 11.564, n = 302, p > 0.021) and the corresponding Jaccard index is 0.118 (p < 0.01), as 9 grid squares had locations for both animals. The grids of spatial association where both occur more frequently (with clumping i.e. darker colours) are given greater weighting than the grids where there may be only one or two fixes for each animal.

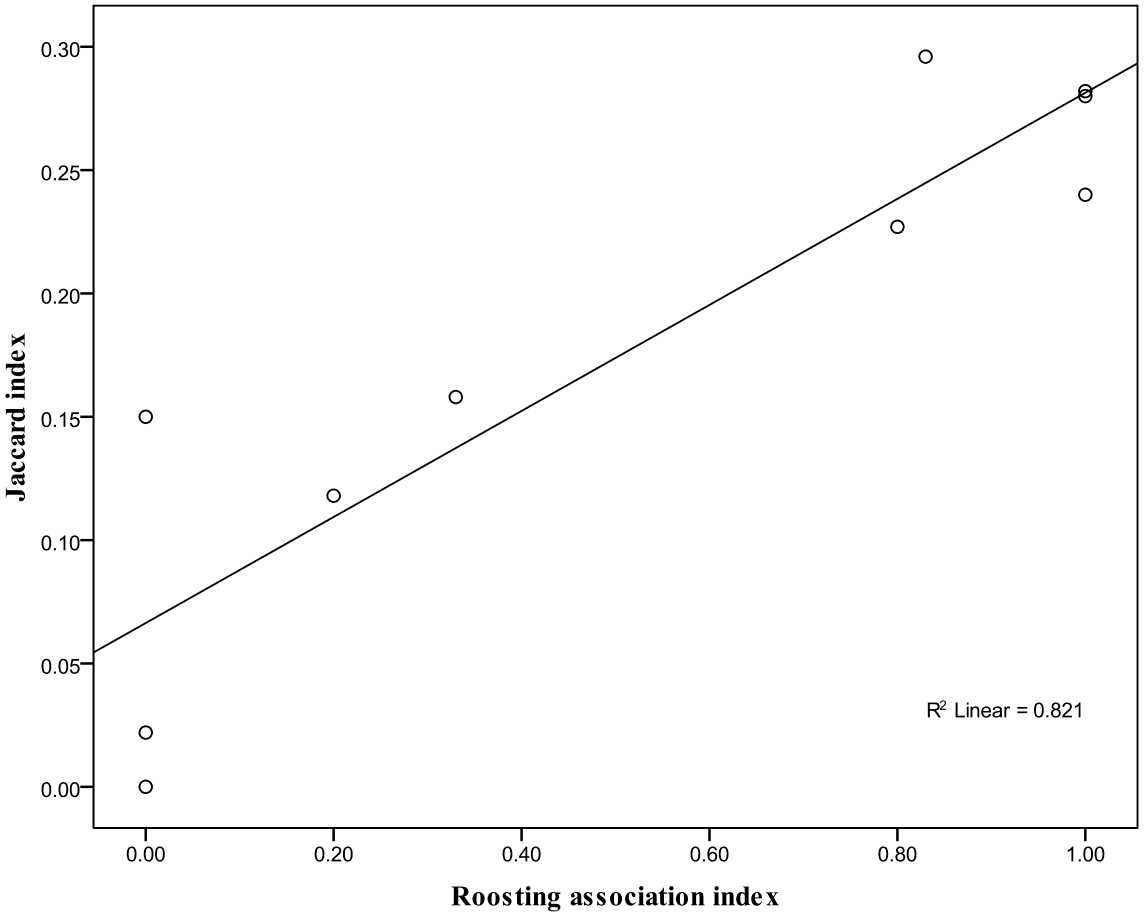


Figure 7.5: Correlation between the roosting association index and overlap of home ranges as measured by the Jaccard index.

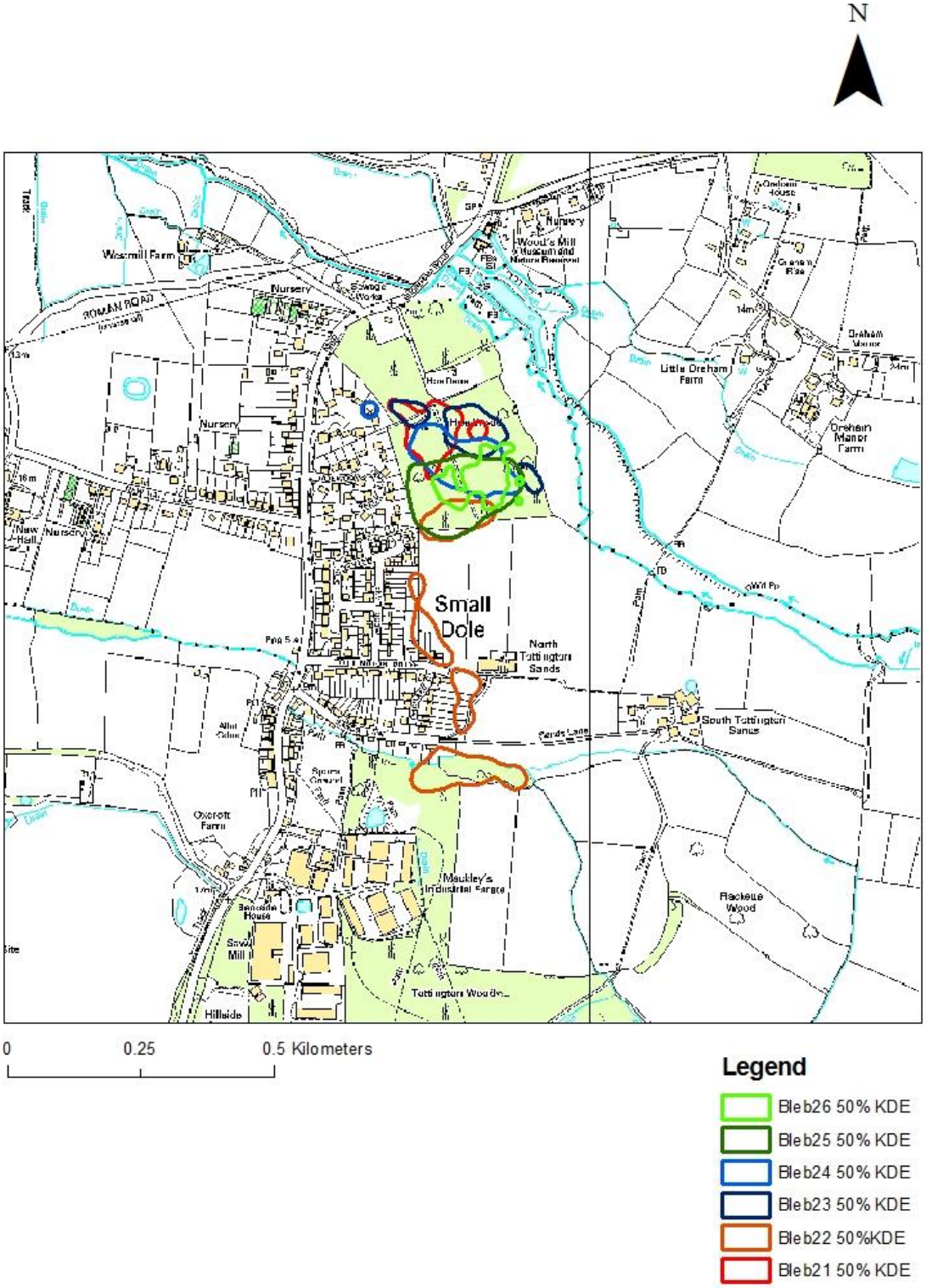


Figure 7.6: Core foraging areas (50% KDE) of six female *P.auritus* radio-tracked from 14th July 2008 to the 11th August 2008 at Hoe Wood.

Species	Habitat	Number of bats radio-tracked	Home range estimator	Mean Range size (ha)	Overlap (%)	Radio-tracked simultanously	Radio-tracked majority of colony	Source
Tent- making bat *Artibeus watsoni*	Primary and secondary tropical forest	42: 26♀ 16♂	100% MCP	3.6	47%	Yes	All individuals in known roosts	Chaverri, G., et al. (2007a); Chaverri, G., et al. (2007b)
Greater sac-winged bat *Saccopteryx bilineata*	Primary and secondary tropical forest	16: 8♀ 8 ♂	100% MCP 95% Kernel 50% Kernel	22±17; 12±6; 1.7±0.9	57 ± 22 (females overlapped with males)	Yes - ♂/♀ dyads	No - only male - female pairs from eight known roosts	Hoffman et al (2007)
Common pipistrelle *Pipistrellus pipistrellus*	Agricultural with riparian woodland	14♀	100% MCP Foraging MCP 75% Kernel	c.200; c.175 c.25	c.36 c.27 c.11	No	No	Nicholls & Racey (2006)
Soprano pipistrelle *Pipistrellus pygmaeus*	Agricultural with riparian woodland	12♀	100% MCP Foraging MCP 75% Kernel	c.60; c.35 c.15	c.24 c.18 c.12	No	No	Nicholls & Racey (2006)
Egyptian slit-faced Bat *Nycteris thebaica*	Acacia savanna with patches of riparian forest	18: 8♀ 8 ♂	95% MCP	13.7	49%	No	No	Monadjem et al (2009)
Barbastelle *Barbastella barbastellus*	Deciduous oak forest interspersed with meadows and brooks	12♀	95% Kernel 50% Kernel	403 67	13 3.2	4 dyads	No	Hillen et al (2009)
Bechstein's bat *Myotis bechsteinii*	Mixed deciduous Beech forest	12♀	100% MCP	21	12.3	Yes - dyads	c.50%	Kerth et al (2001)
Long-tailed bats *Chalinolobus tuberculatus*	Temperate rainforest	50: 28♀ 10 ♂ 12 juvenile	100% MCP	1589 ♂, 1361 post-lac ♀, 657 non-repro ♀	31-68	No	c.50%	O'Donnell 2001
Brown Long-eared bats *Plecotus auritus*	Broadleaved deciduous woodland	24♀	100% MCP 95% Kernel 50% Kernel	51±5; 53±6; 47±6		8 dyads	No	This study

Table 7.5: Studies reporting home range overlap in bats by means of radio-telemetry. Only one study on the Tent-making bat *Artibeus watsonii* radio-tracked all individuals within a known roost. None of the studies radio-tracked all bats present so the range overlap values presented are likely to be an incomplete picture.

8.1 – Conclusions from this study

The aim of this thesis was to examine the structure and function of social calls in Brown Long-eared bats *Plecotus auritus*. Some studies have described the structure of specific *P. auritus* call types from sonograms (Ahlen, 1981; Furmankiewicz, 2004). However, only one study to date has examined the function of *P. auritus* social calls, and this investigated at isolation calls given by infants separated from their mothers (Defanis and Jones, 1995). However, bats produce a variety of different calls in different contexts (Pfalzer and Kusch, 2003) that are likely to have a variety of functions. This research set out to investigate some aspects of social call structure and function using a combination of recording and playback techniques.

The first step was to characterise a range of calls given by *P. auritus* in the vicinity of maternity roosts. Social calls are given very frequently in this context. Calls were systematically recorded across 20 summer maternity colonies in Sussex and the acoustic variation was examined. Initial visual examination of sonograms revealed that there were three distinct groups of social calls which could be reliably distinguished on the basis of their gross structure. Almost 97% of the calls had a similar basic structure, an FM sweep descending in frequency from (mean values) 52kHz to 14kHz, ending with a short (1 ms) QCF, typical of the *P. auritus* social calls described by Ahlen (1981), Russ (1999) and Furmankiewicz (2004). Calls with this similar basic structure were subsequently termed 'Type A' calls. When the acoustic parameters of Type A calls were extracted, model-based cluster analysis revealed that this group could be subdivided into six clusters. These were subsequently named calls one to six. Markov chain analysis revealed that there was no evidence that these call were emitted in a specific order, only that similar call types (calls

from the same cluster) tended to be repeated. However, the Markov chain analysis conducted in this study was limited to the analysis of couplets and it may be that higher order sequences do exist.

The remaining (approximately 3%) calls had a different gross structure and these calls were termed Call 7 and Call 8. Call 7 was characterised by an ascending FM sweep followed by a descending sweep. Call 8 was characterised by a descending FM sweep followed by an ascending FM sweep. Both of these call types showed a showed a linear increase in occurrence throughout the seasons and were also recorded significantly more frequently in September. This increase in occurrence was greater than the increase for any of the Type A calls in September. Call 7 had the same structure as the Autobat output 'stimulus C' (Chapter 4). Responses of *P. auritus* to this stimulus also occurred significantly more frequently in September. These seasonal patterns suggest that the function of the call may be seasonally specific. One explanation would be that these calls are related to mating. Burland et al., (2001) found that male *P. auritus* resident in maternity colonies are philopatric. However, most offspring are fathered by males from outside the colony and it may be that sexually active non-resident males begin to visit maternity roosts at the onset of the mating season. The patterns of vocal production at maternity roost sites (Chapter 3) showed an increase in the number of social calls at roost sites in September, despite the decrease in the number of bats present in the colony. This suggests that there are fewer bats vocalising more frequently, and the roosts could, perhaps, be utilised as mating roosts when the maternity colonies begin to disband. Furmankiewicz (2002; 2008) also described this call type at *P. auritus* underground swarming sites in Poland in early spring when *P. auritus* mate after winter hibernation.

It was often clear from sonograms that more than one bat was producing social calls. It may be that one function of calling around the roost is to strengthen social bonds between members of the colony. This pattern of behaviour may account for the observed correlation between colony size and the number of social calls recorded. The fact that social calls were recorded significantly more frequently in the quarter of the night before sunrise also supports this hypothesis.

There was also no evidence to suggest that certain call types were specific to individual roosts or geographic locations. All of the six Type A calls were recorded at all of the 20 maternity roost sites. Calls 7 and 8 were recorded at most of the maternity roosts across all of the three locations. Therefore, it was concluded that these types of calls could not be roost or location specific. However, this study did not analyse within-call differences and there may be differences in acoustic parameters at a finer scale between roosts and populations.

In the majority of the thesis, I have presented the results of responses to simulated social calls produced by the Autobat to answer questions about the function of *P. auritus* social calls. (Chapters 4, 5, 6 and 7). One of the central questions of the thesis was 'why do bats respond to the Autobat'? Playback is the technique of re-broadcasting natural or synthetic signals to animals and observing their response (McGregor 1992). Studies researching the vocal communication in animals have used playback as a tool to investigate both vocal recognition of conspecifics (Harrington, 1986; Insley, 2000) and call function (Harrington, 1987; Barlow and Jones, 1997). However, playback studies in the field also have limitations in that the responses given are dependent not only on the signal itself but also on the context including both social and ecological factors (McGregor 1992). Playback studies often broadcast the calls more frequently than they would occur under natural conditions in order to elicit a

response from the focal animals, and this use of playback has been referred to as 'super-stimulus'. For example, a study on three bat species that investigated the influence of echolocation call design and foraging strategy on the role of echolocation calls in communication used a playback of 51 repeated feeding buzzes as a 'super-stimulus' (Balcombe and Fenton, 1988).

The Autobat output can also be regarded as a 'super-stimulus' as it repeats sequences of sweeps at much higher rates than they have been recorded in the field. However, if the Autobat output was modelled to occur at a rate that resembled the normal frequency of occurrence of social calls, then it would be extremely difficult to obtain sufficient results with respect to the frequency of response due to very low frequency of calling and the highly mobile nature of bats. However, it raises the possibility that the bats' response may have nothing to do with the similarity of the stimulus to a conspecific's social calls, but that they may be responding to ultrasonic disturbance in the environment. This was tested by the capture experiment (Chapter 4), that compared the bat's responses to simulated calls of their own species, another species and mechanical noise of a comparable duration and bandwidth. *P. auritus* were captured significantly more frequently to simulations of their own social calls. This suggests that the response of *P. auritus* to the stimulus is a social one, elicited by the similarity of the stimulus to conspecific social calls.

Once I had established that the similarity of the stimulus to a *P. auritus* social call influenced the propensity of bats to respond, I then (Chapter 4): (i) assessed whether the responses varied seasonally and between sexes; and (ii) analysed the nature of behavioural responses elicited by a range of stimuli modelled on various social calls of their own species. There was

evidence of a seasonal difference in the responses to different stimuli whereby high repetition high intensity calls elicited more responses earlier in the season (June) compared to later in season (August / September). Females were captured significantly more frequently earlier in the season compared to later when males were caught more than females. Furthermore, the behavioural responses observed on infra-red video in June were more complex, longer in duration, and occurred more frequently than later in the year.

One of the hypotheses put forward to explain the bat's response to the stimulus is that the responses are related to defence of the foraging area. In order to test this hypothesis, the activity patterns and the extent of home range use was ascertained for 24 female *P. auritus* across a variety of woodland sites in Sussex (Chapter 5). The study subsequently examined the responses of females in relation to home range use by varying the stimulus location within the bat's foraging range (Chapter 6). A novel technique was used to identify responding individuals and the study found that females were more likely to respond to simulated social calls played in their core foraging range than in their peripheral foraging range or outside of their range. The propensity of *P. auritus* to respond maximally to simulated social calls in core foraging areas is consistent with the resource defence hypothesis.

One alternative explanation to the resource defence hypothesis is that the response to the stimulus represents coordination of foraging by attracting other colonies members to resources. Social calls can serve to attract and recruit conspecifics to food (Wilkinson and Boughman, 1998; Pollick et al., 2005; Dechmann et al., 2010). This hypothesis was explored by examining overlap in range use amongst females radio-tracked in the same woodland and, by also assessing the interactions of pairs of bats by simultaneously radio-tracking 10 female

dyads. There was extensive overlap in range use for adjacent pairs of females radio-tracked at the same woodland site. Given that only a very small proportion of each of the colonies was radio-tracked at each site, the results suggest that females are likely to share with multiple other females. The interaction analysis indicated that most adult female pairings were neither attracted to nor avoided one another when foraging, but adult–juvenile pairings were positively associated more frequently than would be expected by chance. Furthermore, dyads that had greater overlap in their ranges, also roosted together more frequently.

Adult females from the same colony may be aware of one another's presence when foraging and may be able to discriminate colony members from intruders. There was, however, no evidence of roost specific call signatures (Chapter 3). Even though the location within the foraging range from which the stimulus is broadcast clearly influenced the propensity of females to respond, based on the results of this study it cannot be completely ruled out that the response to stimulus represents co-ordination of foraging. *P. auritus* females may forage alone for most of the time but show co-operative foraging tendencies by calling to advertise a rich food source, which attracts one or more conspecifics. If that were the case, however, it may be expected that there would be no difference in response in relation to whether the call came from the core or the peripheral foraging area.

A further question which is important to address is whether the Autobat is a legitimate way of conducting research on the function of bat social calls. The use of synthesised calls produced by the Autobat has some distinct advantages over the playback of recordings of social calls. It is extremely difficult to get high quality recordings of brown long-eared bat social calls away from roosts, as they tend to produce social calls infrequently and unpredictably. This means that calls must be recorded opportunistically and recording conditions tend to be poor, with

weak signals and excessive background noise. While such recordings are not suitable for playback, as long as the time and frequency coordinates of the call can be accurately measured from a sonogram, the calls can be used to produce very clear output from the Autobat. Also, the use of artificial stimuli allows for the control of differences between stimuli and avoids potential problems of pseudo-replication inherent with playback studies (for review see Kroodsma et al., 2001). Furthermore, the equipment required for playback is also more expensive and fragile than the Autobat, making it more difficult to use under field conditions. This is especially important for experiments that require frequent relocation of the equipment in woodland at night.

On the other hand there are potential drawbacks to using synthesised call stimuli. The main one is that the synthesised calls are artificial sounds, and it may be that differences in acoustic quality between the stimuli and actual social calls influence the way that bats respond to them. However, similar problems may well occur with playback of recorded vocalisations. Even with flat response microphone and speaker, it is likely that there will be acoustic differences between the original call and the reproduced call. One is that the recorded call will have undergone some attenuation between bat and microphone. The playback output will then undergo further attenuation between the speaker and the bats that hear it, and so the intensity of playback will be reduced, and particularly that of the high frequency portions of the call. Also, naturally emitted calls are given by bats that are in flight, and so the sound source is moving, not only in terms of whole body movements, but also movements of the head in relation to the body. This is likely to alter sound quality in ways that will not be simulated by a fixed speaker.

8.2 – Suggestions for future research raised by this study

This has been the first study to attempt to quantitatively classify a variety of social calls of *P. auritus* and investigate responses to them. However, one of the problems with conducting a field study in this manner is that there is very little data on the identity of the caller and, also, it was often not known who was responding to the stimulus calls (with the exception of the individuals that were marked; Chapters 4 and 5). In a laboratory setting this aspect could be controlled for by recording callers and watching the responses of known individuals. The following discussion examines how each of the key areas I researched could be expanded upon and improved.

(i) It was possible to objectively classify individual social calls by recording calls at the roost sites and subjecting these calls to statistical classification techniques. However, based on recordings from the field, it is likely that individuals often produce social calls in a sequence, as opposed to singularly. Nonetheless, the first order Markov chain analysis did not find any evidence that one particular call type followed another type. In Chapter 3, however, it was demonstrated that the number of social calls at roost sites was significantly correlated with the number of bats roosting in the maternity colony and one explanation for this trend could be that the majority of bats are contributing to the vocalisations. Consequently, interpretation of whether calls are produced as sequences of vocalisations is difficult because multiple vocalisations from different individuals would confound the results. More controlled studies than are possible in the field using a laboratory based approach may be required in order to fully investigate and quantify whether *P. auritus* produce vocalisation as sequences, as demonstrated for other species of bat by researchers under laboratory conditions (Kanwal et al., 1994; Bohn et al., 2009).

(ii) Experiments with a temporary captive colony of bats could also address the question of whether the Autobat is a legitimate manner in which to conduct research on bat social calls. The best way to assess whether the artificial nature of synthesised or playback calls is influencing the nature of the bats' responses would be to observe their responses to naturally emitted calls. This would be impossible to do in a field situation with free-flying bats, but it may be possible to gain some insight from observation with bats in captivity, if they produced social calls sufficiently frequently. This would also allow for experimental comparison of bats' responses to output from the Autobat and playback of recorded social calls.

(iii) With bats in temporary captivity it would also be possible to examine whether individual identity is encoded within social call structure. Individual recognition is important for roost members to recognise roost associates. If males are present in roost then it is important to recognise resident males from males of adjacent populations. If, for example, the Autobat were used to elicit vocal responses from known captive individuals, and these responses were recorded, these could then be played back to subjects to examine how responses differ to different kinds of calls. It would also be possible to examine how responses differ to calls of roost mate and non-roost mates. This could also be combined with DNA analyses to examine whether responses to kin differ from those to non-kin colony members. By using infra-red video and ultrasound recording it would be possible to see how colony members of both sexes approach the stimulus.

(v) An alternative hypothesis that could be examined as to why bats vocalise at roosts sites, and in particular vocalisations in the period before dawn, could be that social calling is related to the group decision making process with respect to roost choice. Studies have demonstrated that bats roosting in trees frequently switch roost sites (Lewis, 1996; Russo et

al., 2004; Willis and Brigham, 2004; Vonhof and Betts, 2010), and radio-tracking *P. auritus* roosting in trees in Sussex also demonstrated that this species frequently switched roost during the radio-tracking period (Murphy et al in prep). Although the social calls recorded in this study were from bats roosting in buildings, in the absence of human influence *P. auritus* would have roosted in trees and vocalising at roost sites may be inherent in the group decision-making process of deciding on the tree in which to roost. Using vocalisations in the context of movement decisions has been demonstrated for group living species such as meerkats *Suricata suricatta* (Bousquet et al., 2011), primates (e.g. white-faced capuchin, *Cebus capucinus* (Boinski and Campbell, 1995)), and birds (e.g. green woodhoopoes *Phoeniculus purpureus* (Radford, 2004)). It would be instructive to examine if there were differences between calls at synanthropic roosts and tree roosts. An intensive study of tree-roosting colonies, which switch roosts very frequently, may help to elucidate the function of calls in relation to roost switching. This could be achieved using a combination of radio-tracking, recording behaviours with infra-red video and recording their vocalisations.

(vi) Call 7 were frequently recorded at maternity roost sites later in the season (September) compared to earlier in the season (May – June). They had the same call structure as stimulus C (ascending and descending FM sweep) to which bats responded significantly more frequently at foraging sites. One hypothesis proposed for this call type is that the call may be related to mating and an experiment that could specifically address this hypothesis would be to playback simulations of this call type and to capture the individuals responding. Comparing the sex ratio and whether the males responding were sexually mature may indicate whether the calls play a role to function in mate attraction. It would also be interesting to ascertain whether there is sexually mature non-philopatric males present at maternity roost sites in September and how, if at all, these males are related to females

present in the colony in September. If summer maternity colonies also function as mating colonies for *P. auritus* this may have important implications for their conservation.

(vii) One suggested explanation for bats response to the stimulus calls was that the responses represent defence of resources. This hypothesis was supported by the result that females responded maximally to the stimulus in their core foraging area. If the resource defence hypothesis is true then it would be interesting to examine whether females maintained this response over time and if their ranges and / or responses to the stimulus changed seasonally or even between years. Resources availability may change from season to season or from year to year, prompting animals to shift their home range, make seasonal excursions, or alter patterns of social organisation (Lott, 1991). Radio-tracking a number of individuals repeatedly, within and between years, to ascertain how or if their home range changes will provide insight into how fixed or flexible the system is.

(viii) Although there was little conclusive evidence from the radio-tracking data that adult females dyads were interacting when foraging, one way to further investigate whether there is any evidence of co-ordinated responses to the stimulus would be to try to catch three or four females in the same area, ascertain their home range, and to then present them with stimulus calls in relation to their home range use and investigate whether there is any co-ordination in responses using a combination of radio-tracking and infra-red video. This approach would require a number of field-workers to simultaneously radio-track and locate females, but it is certainly a feasible approach given adequate resources. If only one female responds, the female who primarily occupies that part of the range, then this could indicate that the female is defending resources within her range. If a female shares her range with one or more females, do all females respond?

Finally, on a practical note, understanding why bats respond to the stimulus is important for developing the system as a survey tool. In the UK, for example, the Autobat is currently used by the Bat Conservation Trust to systematically survey for Bechstein's bat *Myotis bechsteinii* and has also been used for systematic species surveys and experiments in other parts of Europe (Goiti et al., 2008; Boston et al., 2010; Schöner et al., 2010). The data and experiments detailed in Chapters 4 and 6 demonstrated that *P. auritus* rapidly approach the source of the stimulus broadcast. However, repeatedly broadcasting simulations of social calls may inadvertently affect an individual's fitness by deterring it from its preferred foraging locality. Therefore, it is important that the use of the Autobat as a survey tool is conducted in a controlled manner whereby targeted areas are not 'over-surveyed'.

Although the work carried out in this thesis has shed some light on the function of social calls in *P. auritus*, many further issues remain to be investigated. More controlled studies than are possible in the field may be required if the functions of social calling are to be thoroughly investigated and quantified.

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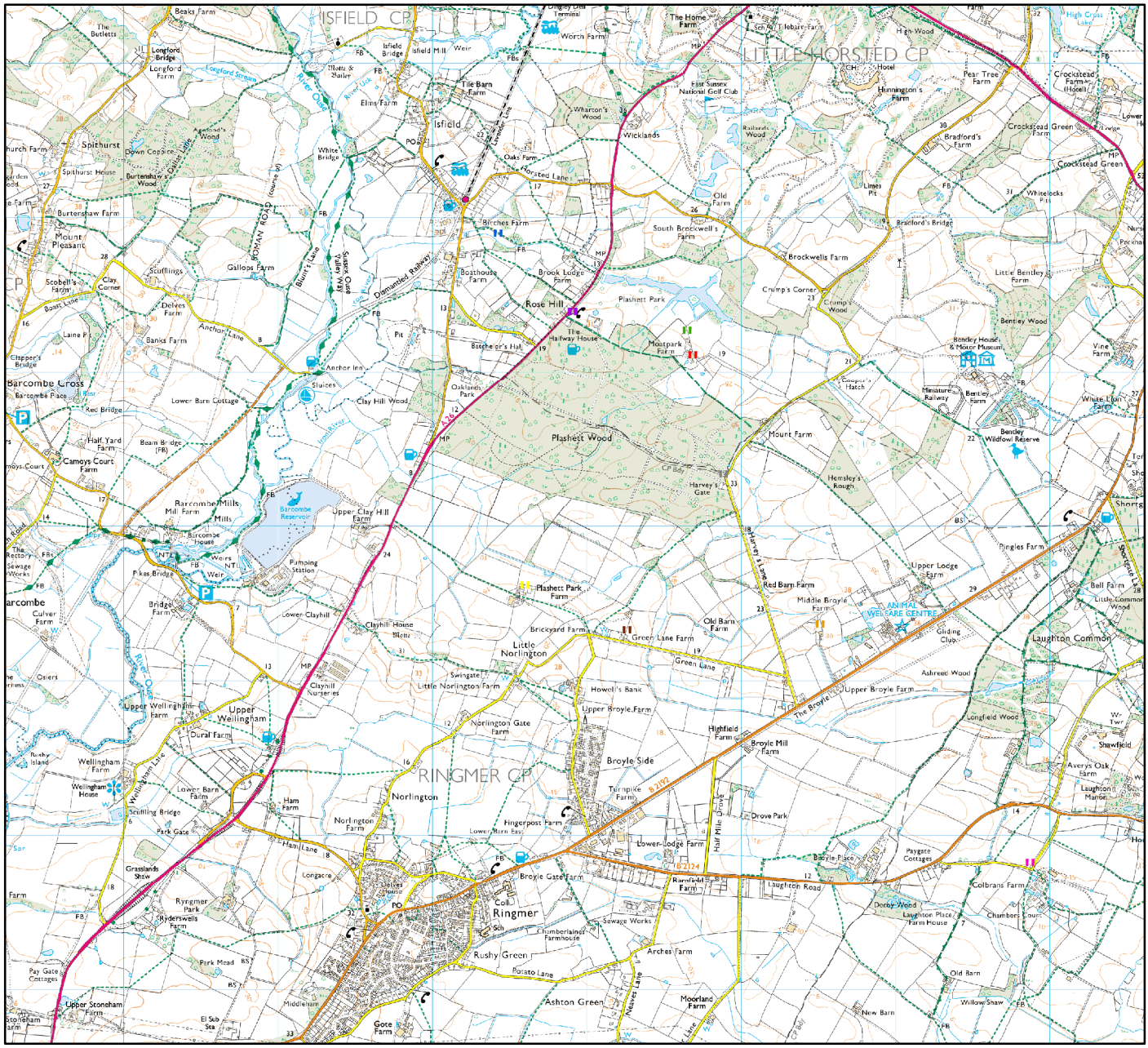
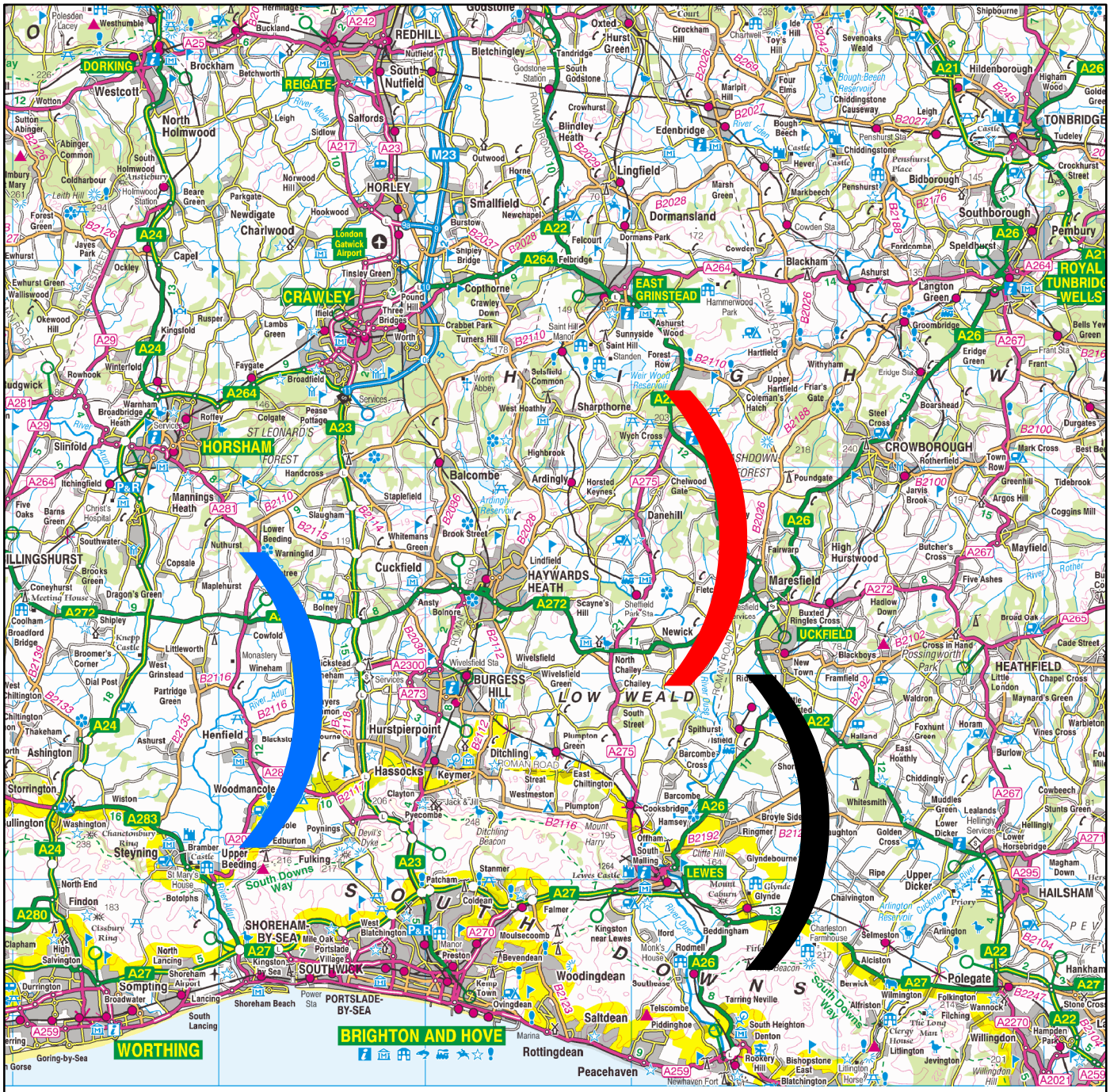


Legend

- B1: Middle Broyle Farm
- B5: Plashett Park Farm
- B6: Harveys Gate
- B8: Moat Park Farm
- B10: Colbrans Cottages
- B20: 2 Plashett Park Gate
- B21: 3 Plashett Park Gate
- B23: Moat Park Cottage
- B26: Green Lane Farm
- B28: Oast House Isfield

Appendix A.3.1: Roosts around the Plashett Wood Area North of Lewes.
Two roosts, 2 & 3 Plashett Park Gate, are situated in the same row of cottages (but are not physically connected). These roosts are denoted by the one purple marker.



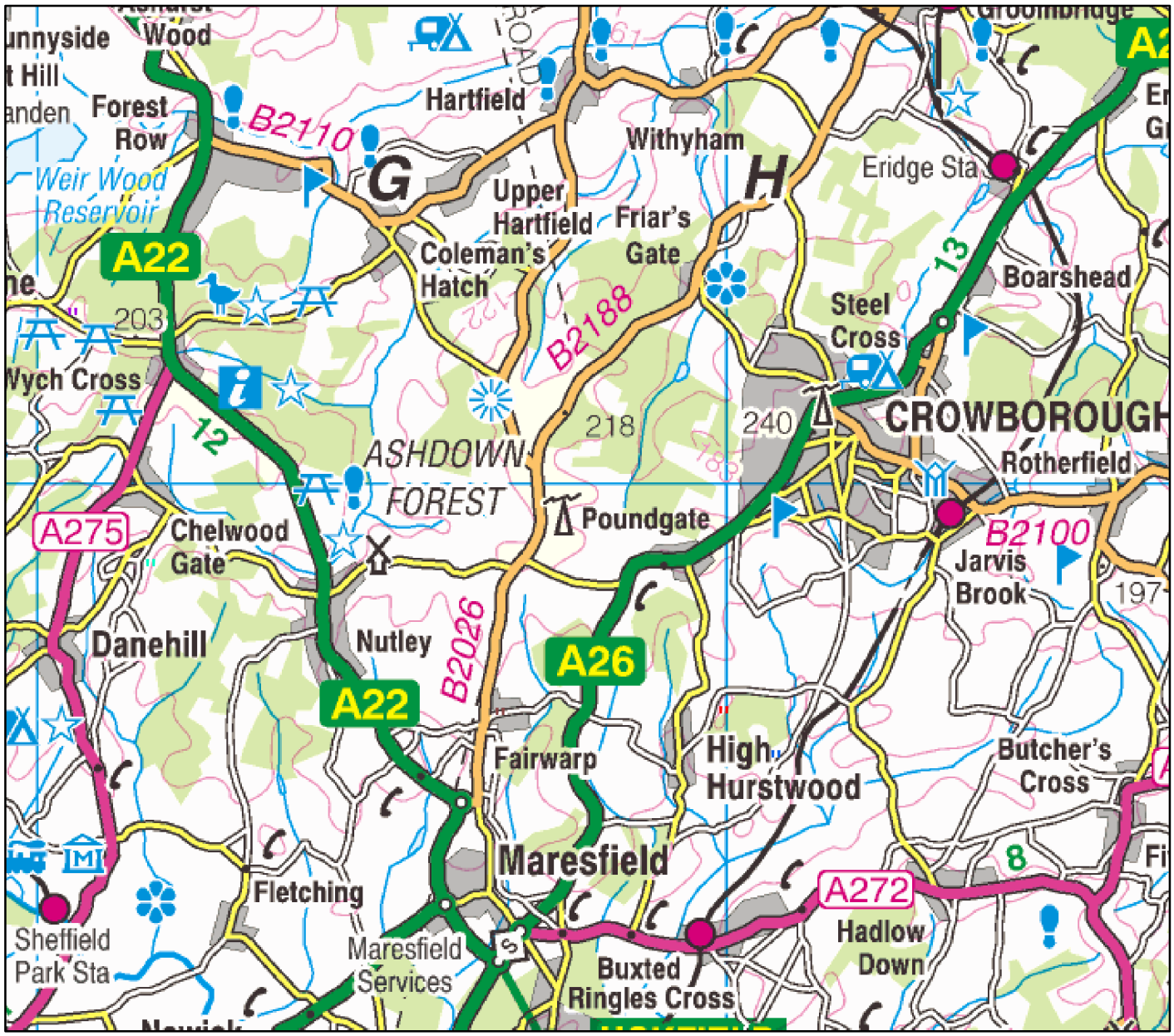
Legend

) **Area 1: East Sussex North-east of Lewes**

) **Area 2: East Sussex Ashdown Forest**

) **Area 3: West Sussex Cowfold Area**

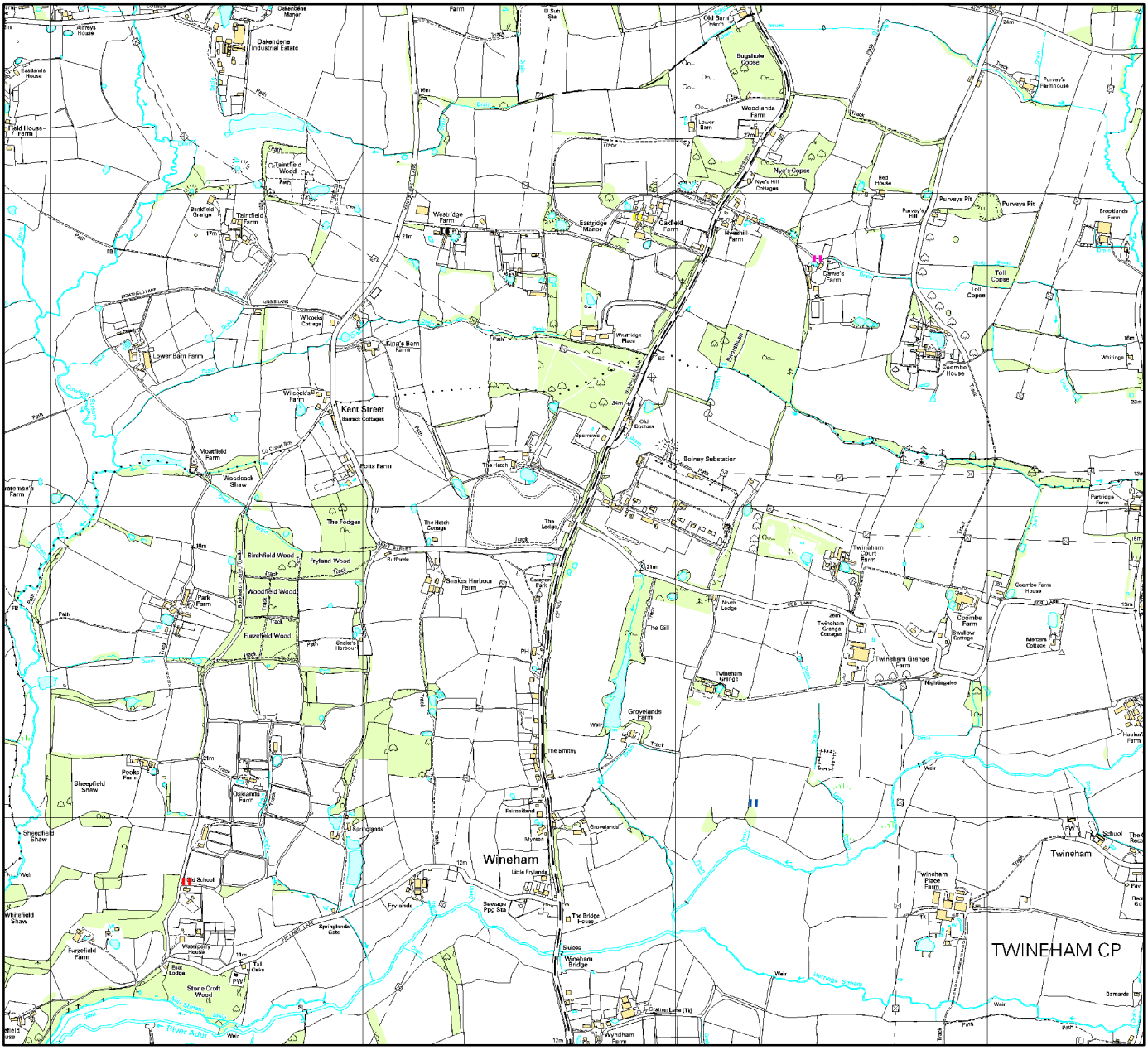
Appendix A.3.2: The three areas in Sussex identified for the study of Brown Long-eared social calls at roost sites.



Legend

- B2: Sleeches Farmhouse
- B4: Pickreed Cottage
- B9: Lantern Cottage
- B13: Charlwood Gate
- B16: Springfield Cottage

Appendix A.3.3: Locations of Brown Long-eared roosts in the Ashdown Forest Area, East Sussex, used in the study.



0 0.25 0.5 1 Kilometers

Legend

- B3: Oakfield
- B7: Kings Kent
- B15: Old School
- B22: Oakwood Farm House
- B27: Dawes Farm

Appendix A.3.4: Locations of Brown Long-eared roosts in the Cowfold area, West Sussex used in the study.

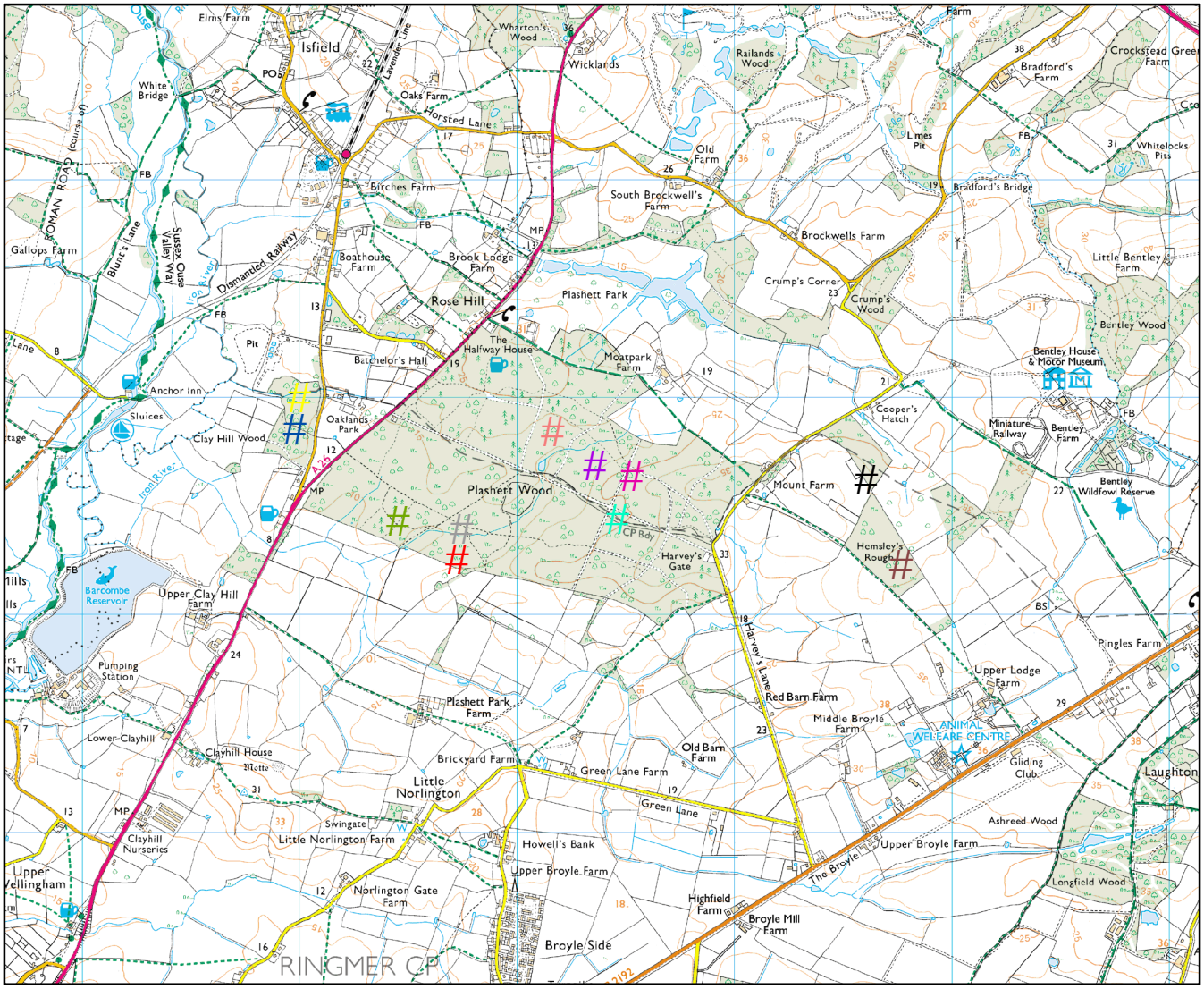
Roost ref		**Date**		**Sunset**		**Sunrise**	
Observer		**Exit hole**		**Moonrise**		**Moonset**	
Temp start		**Temp end**		**Mean max**		**Mean min**	
Humid start		**Humid end**		**Cloud cover**		**Rain**	
Wind		**Time start**		**Time finish**		**Aspect**	
Time of first exit							
Time of last exit							
Time in 10 minute intervals							

	Bats out	**Bats in**	**Total**
1			
2			
3			
4			
5			
6			
7			
8			

Comments

Sketch of exit site plus direction of flight path

Appendix A.3.5: Roost emergence/re-entry recording sheet



0 1 2 Kilometers

Legend

1
2
3
4
5
6
7
8
9
10
11

Appendix A.4.1: Eleven capture locations used at three woodland sites.

Appendix A.4.3: Measuring intensity of the six stimuli

The intensity of the six stimuli was measured in a three stage process. Firstly, the frequency response of the transducer (i.e. the Autobat speaker) was obtained using the following method. A free field microphone (Bruel & Kjaer model 4191 with a flat response up to 40kHz), was placed inside a standard sound source (calibrator Bruel & Kjaer model 4230, 94dB Spl at 1kHz). The microphone's output was amplified by 40dB which was then measured as -1.4dB on a dB meter. However, since the transducer could not be placed directly inside the calibrator, the standard sound source was subsequently moved away from the free field microphone, to a distance of 2cm, which resulted in a reduction of the microphone output to -3.2dB. The standard sound source was then replaced with the transducer at a distance of 2cm and an input from a signal generator (constant level 7.4 volts p-p) was applied while sweeping through the frequency range 1kHz to 60kHz, in 1kHz steps, in order to determine the frequency response of the transducer (See Table A.4.2.1 column 1 for detailed measurements.).

The second stage of the process was to calibrate the microphone of the recording system, the Ultrabat, using the, now calibrated, Autobat transducer as a signal source. The transducer was placed 2cm from the microphone (Knowles EK23132) of the Ultrabat recording system. A constant level signal of 7.4 volts p-p from the signal generator (Venner TSA 625/2) was applied to the transducer. As this level of 7.4 v p-p caused overloading of the recording system, it was reduced by 25dB to prevent overload occurring. This signal was swept through the frequency range of 1khz to 60kHz in 1kHz steps. From the analysis of these recordings it was possible to determine the sound pressure level, (shown in column 2 Table A.4.3.1).

The final stage in the calibration stage was to measure the frequency which produced the highest signal level of the 6 Autobat stimuli calls A – F in Bat Sound. Calls A, D and F were comprised of one call type only, call C comprised of two and calls B and E comprised of three different call types. Once a sound level measurement for the peak frequency was obtained for each of the calls (from the spectrogram in Bat Sound), the Spl that the transducer produced to give this level could be determined using the measurements obtained for the Autobat transducer (Figure A.4.3.1) and Ultrabat recording system calibration (Figure A.4.3.2). So, for example, call A had a peak frequency of 32.7 kHz, -27dB in Batsound. At 33kHz the calibrated microphone gives produces -6dB. The Autobat transducer produces 71.7dB at 33kHz. Therefore, call A at 33kHz produces 50.7 SPL dB (- 6dB less than -27dB is 21dB, 21dB less than 71.7dB gives a reading of 50.7 SPL dB). Amplified calls increased the SPL by – 3dB.

Column A

BK mic (close in)	**SPL (dB)**	**BK mic at 2cm**	**SPL (dB)**
-14	94	-32	76
Frequency (kHz)	**Transducer at 2cm**	**SPL dB**	**SPL dB -25**
1	-53.2	54.8	29.8
2	-50.7	57.3	32.3
3	-44.7	63.3	38.3
4	-41	67	42
5	-35.7	72.3	47.3
6	-32.8	75.2	50.2
7	-30.4	77.6	52.6
8	-28.4	79.6	54.6
9	-26.9	81.1	56.1
10	-25.3	82.7	57.7
11	-25.2	82.8	57.8
12	-24.5	83.5	58.5
13	-22.9	85.1	60.1
14	-22.7	85.3	60.3
15	-23	85	60
16	-22.8	85.2	60.2
17	-22.2	85.8	60.8
18	-21.5	86.5	61.5
19	-21.4	86.6	61.6
20	-21.2	86.8	61.8
21	-20.5	87.5	62.5
22	-17	91	66
23	-15.5	92.5	67.5
24	-14.5	93.5	68.5
25	-15.7	92.3	67.3
26	-15.6	92.4	67.4
27	-15.1	92.9	67.9
28	-13.7	94.3	69.3
29	-9.9	98.1	73.1
30	-10.7	97.3	72.3
31	-10.4	97.6	72.6
32	-11.4	96.6	71.6
33	-11.3	96.7	71.7
34	-10.4	97.6	72.6
35	-9.2	98.8	73.8
36	-7.5	100.5	75.5
37	-8	100	75
38	-8.3	99.7	74.7
39	-8.3	99.7	74.7
40	-8.6	99.4	74.4

Column B

Microphone callibration	
Frequency (kHz)	**SPL Db**
20	-16.2
21	-14.5
22	-13.6
23	-11.1
24	-9.4
25	-6.4
26	-5.1
27	-5.1
28	-7.7
29	-7.7
30	-6.8
31	-1.7
32	-2.6
33	-6
34	-5.5
35	-4.3
36	-1.7
37	-1.7
38	-3.4
39	-4.3
40	-6.1
41	-6
42	-3.4
43	-0.9
44	-3.4
45	-4.3
46	-6
47	-6
48	-2.6
49	-3.4
50	-6
51	-6.2
52	-6.2
53	-3.4
54	-3.4
55	-4.3
56	-4.3
57	-5.1
58	-6
59	-10.2
60	-14.5

CONT....

Frequency (kHz)	Transducer at 2cm	SPL dB	SPL dB -
44	-11.7	96.3	71.3
45	-13.5	94.5	69.5
46	-13.9	94.1	69.1
47	-13.5	94.5	69.5
48	-13.4	94.6	69.6
49	-13.5	94.5	69.5
50	-13.1	94.9	69.9
51	-12.2	95.8	70.8
52	-12	96	71
53	-13	95	70
54	-13.3	94.7	69.7
55	-14.6	93.4	68.4
56	-15.3	92.7	67.7
57	-14.9	93.1	68.1
58	-14.3	93.7	68.7
59	-14	94	69

Table A.4.3: Frequency response of transducer, column 1 and the Ultrabat recording system microphone, column 2.

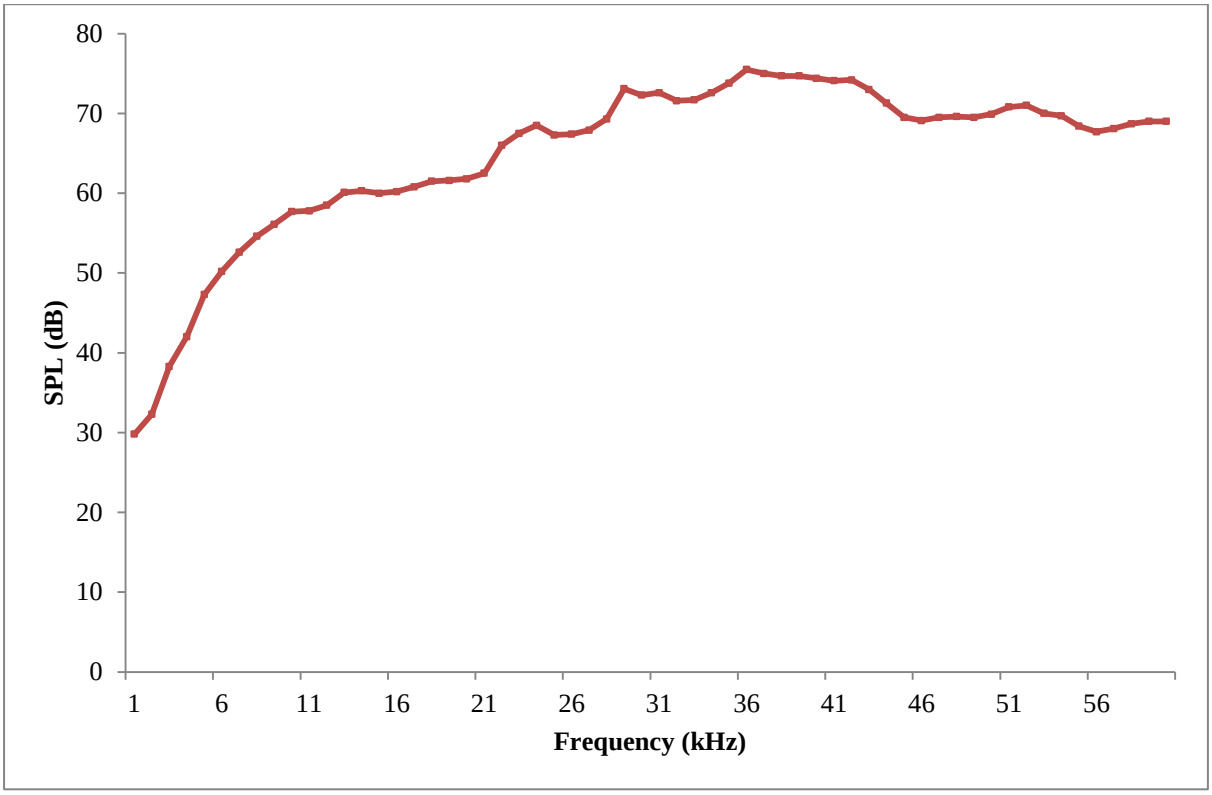


Figure A.4.3.1: Frequency response curve of Autobat transducer.

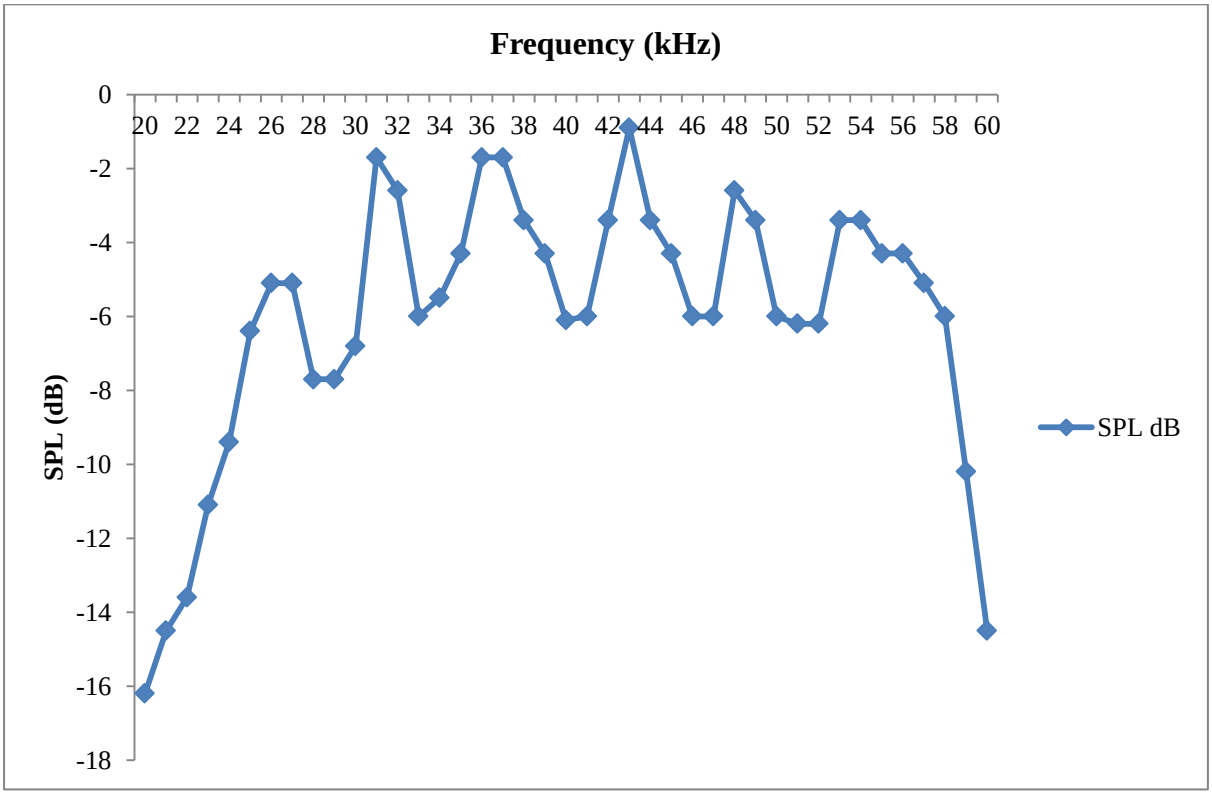


Figure A.4.3.2: Frequency response curve of Ultrabat microphone.

Behavioural definitions for categorisation system

Approach stimulus	The responding bats(s) directly approach the speaker.
Fly near	The responding bats(s) approach the stimulus and subsequently fly near to the stimulus.
Continuous Flight	The responding bat(s) flies in a direct line without deviating from a straight flight path.
Deviated Flight	The responding bat(s) changes direction of flight either on approaching or departing from the stimulus location.
360^{o} rotation	The bat(s) fly around the stimulus in a circular motion completing at least one full 360^{o} rotation.
Moving continuously	The bat(s) continuously flies near to the stimulus without pausing to investigate the sound source and without completing a full 360^{o} rotation.
Hover	The bats(s) pause in their flight to stay suspended in the air fluttering near one place.

Appendix A.4.5: Definitions of terms used in behavioural binary key.

Pair	Bat	Site	MCP 100% (ha)	KDE 95% (ha)	KDE 50% (ha)	% Overlap 100% MCP with simultaneously tracked female	% Overlap 95%KDE with simultaneously tracked female	% Overlap 50%KDE with simultaneously tracked female
1	9	Clayhill	12.9	6.1	2.0	23.4	43.3	55.5
	10	Clayhill	6.4	4.1	2.0	50.8	97.9	87.7
2	13	Hemsleys	5.1	4.0	1.8	55.8	52.4	45.3
	14	Hemsleys	6.1	3.9	2.3	55.9	50.1	42.0
3	17	Capite	7.9	3.1	1.1	0.0	0.0	0.0
	18	Capite	5.1	1.7	1.1	0.0	0.0	0.0
4	19	Bignor	6.1	2.6	1.1	60.3	49.5	38.6
	20	Bignor	4.9	3.1	1.5	65.7	45.6	36.0
5	21	Small Dole	3.9	3.1	1.6	2.4	0.6	0.0
	22	Small Dole	8.5	6.1	3.6	0.9	0.3	0.0
6	23	Small Dole	5.1	4.0	2.0	74.7	58.4	41.8
	24	Small Dole	6.6	4.9	1.9	59.1	59.3	42.3
7	25	Small Dole	8.1	7.5	3.7	52.1	62.1	61.4
	26	Small Dole	6.0	4.1	2.5	38.3	82.7	79.9
8	27	Capite	6.1	3.1	2.1	29.1	24.1	17.5
	28	Capite	7.6	4.0	3.0	24.3	25.1	19.2
9	29	Bignor	5.5	3.5	2.0	35.9	44.0	35.1
	30	Bignor	2.7	1.6	0.7	92.3	95.2	96.8
10	31	Bignor	9.9	6.2	2.8	66.8	49.7	30.0
	32	Bignor	8.9	5.1	3.9	31.4	17.3	18.9

Appendix A.7.1: Percentage overlap of each home range estimate for 20 female *P. auritus* radio-tracked simultaneously in pairs.

Clayhill Wood	Bleb 9	Bleb 10	Bleb 11
Bleb 9		23.40	12.17
Bleb 10	50.80		62.73
Bleb 11	15.78	37.37	

Hemsleys Rough	Bleb 12	Bleb 13	Bleb 14
Bleb 12		30.69	43.59
Bleb 13	70.39		55.80
Bleb 14	83.74	55.90	

Capite Wood	Bleb 17	Bleb 18	Bleb 27	Bleb 28
Bleb 17		0	0.95	35.49
Bleb 18	0		0	0
Bleb 27	1.23	0		29.31
Bleb 28	38.12	0	24.30	

Bignor Park	Bleb 19	Bleb 20	Bleb 29	Bleb 30	Bleb 31	Bleb 32
Bleb 19		60.30	13.04	5.16	13.7	33.79
Bleb 20	65.70		30.27	6.76	5.52	36.16
Bleb 29	13.81	29.65		35.90	58.16	71.9
Bleb 30	10.73	13.14	92.30		93.71	100
Bleb 31	7.42	3.62	29.59	24.29		66.80
Bleb 32	4.13	3.45	8.28	5.87	31.40	

Hoe Wood	Bleb 21	Bleb 22	Bleb 23	Bleb 24	Bleb 25	Bleb 26
Bleb 21		2.40	72.75	92.79	40.99	59.82
Bleb 22	0.90		0	3.72	20.74	3.61
Bleb 23	54.72	0		74.70	37.15	58.39
Bleb 24	55.07	5.12	59.10		52.26	65.58
Bleb 25	19.14	22.46	23.07	41.11		52.10
Bleb 26	38.52	5.39	50	71.17	38.30	

Appendix A.7.2: Percentage range overlap of 100% MCP for simultaneously (no colour) and non-simultaneously (shaded in yellow) radio-tracked bats at the same site in the same year.

Clayhill Wood	Bleb 9	Bleb 10	Bleb 11
Bleb 9		43.30	21.27
Bleb 10	97.90		43.61
Bleb 11	31.24	42.91	

Hemsleys Rough	Bleb 12	Bleb 13	Bleb 14
Bleb 12		26.8	24.88
Bleb 13	36.05		52.4
Bleb 14	42.99	50.1	

Capite Wood	Bleb 17	Bleb 18	Bleb 27	Bleb 28
Bleb 17		0	0.05	7.22
Bleb 18	0		0	0
Bleb 27	0.05	0		24.10
Bleb 28	5.73	0	25.10	

Bignor Park	Bleb 19	Bleb 20	Bleb 29	Bleb 30	Bleb 31	Bleb 32
Bleb 19		49.50	14.79	6.96	18.4	6.47
Bleb 20	45.60		22.11	2.37	0.74	2.96
Bleb 29	11.56	20.56		44.0	42.25	1.28
Bleb 30	5.44	2.19	95.20		33.43	0.75
Bleb 31	8.03	0.38	24.13	18.66		49.70
Bleb 32	2.02	1.1	0.51	0.3	17.30	

Hoe Wood	Bleb 21	Bleb 22	Bleb 23	Bleb 24	Bleb 25	Bleb 26
Bleb 21		0.60	66.82	89.52	43.05	36.37
Bleb 22	0.30		0	3.82	23.25	11.13
Bleb 23	53.64	0		58.4	39.46	34.49
Bleb 24	71.87	4.80	59.3		57.03	48.56
Bleb 25	15.68	17.13	17.90	33.42		62.1
Bleb 26	30.20	18.71	15.64	64.87	82.7	

Appendix A.7.2: Percentage range overlap of 95% KDE for simultaneously (no colour) and non-simultaneously (shaded in green) radio-tracked bats at the same site in the same year.

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Clayhill Wood	Bleb 9	Bleb 10	Bleb 11
Bleb 9		55.50	11.79
Bleb 10	87.70		13.46
Bleb 11	11.31	13.5	

Hemsleys Rough	Bleb 12	Bleb 13	Bleb 14
Bleb 12		5.56	8.17
Bleb 13	8.18		45.30
Bleb 14	14.68	42.0	

Capite Wood	Bleb 17	Bleb 18	Bleb 27	Bleb 28
Bleb 17		0	0	0
Bleb 18	0		0	0
Bleb 27	0	0		17.5
Bleb 28	0	0	19.2	

Bignor Park	Bleb 19	Bleb 20	Bleb 29	Bleb 30	Bleb 31	Bleb 32
Bleb 19		38.60	0.10	0.25	4.61	2.76
Bleb 20	36.0		9.32	0	0	3.47
Bleb 29	0.07	5.59		35.10	20.34	0
Bleb 30	0.80	0	96.80		58.48	0
Bleb 31	2.21	0	13.69	8.80		30.0
Bleb 32	0.97	1.03	0	0	18.90	

Hoe Wood	Bleb 21	Bleb 22	Bleb 23	Bleb 24	Bleb 25	Bleb 26
Bleb 21		0	38.54	42.92	5.18	0
Bleb 22	0		0	0	23.51	2.44
Bleb 23	34.29	0		41.80	72.77	3.67
Bleb 24	27.58	0	42.30		52.54	37.91
Bleb 25	2.38	26.45	1.33	37.55		61.40
Bleb 26	0	6.58	4.53	64.84	79.90	

Appendix A.7.4: Percentage range overlap of 50% KDE for simultaneously (no colour) and non-simultaneously (shaded in blue) of radio-tracked bats at the same site in the same year.

Function of social calls in Brown Long-eared bats *Plecotus auritus*

by

Stephanie E. Murphy

Presented for the degree of Doctor of Philosophy in the School of Life Sciences at the University of Sussex

2012

Author and Brown Long-eared bat *Plecotus auritus* at a maternity roost site in East Sussex May 2007.

This thesis is dedicated to my mother Nuala Burnett

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University of Sussex

Stephanie Murphy: Thesis submitted for the degree of Doctor of Philosophy

Summary: Function of social calls in Brown Long-eared bats *Plecotus auritus*

Microchiropteran bats produce vocalisations for two purposes: echolocation and communication. Vocalisations used for communication are often referred to as social calls. In this thesis I examined the nature of Brown Long-eared bats *Plecotus auritus* social calls recorded at roost and foraging sites through a combination of recording and playback experiments.

A total of 11,484 social calls were recorded at 20 maternity roosts sites and three types of vocalisations were identified on the basis of shape, referred to as Type A, B, and C. Although Type A vocalisations shared the same basic pattern, it was a very large group within which there was a lot of variation in acoustic parameters. Principal component analysis and model-based cluster analysis were used to look for patterns within this group, and this identified six clusters. Maternity colonies surveyed in this study varied in size from as few as nine up to 98 bats, and the number of social calls recorded at the roost sites was highly correlated with the numbers of bats present in the colony. The analysis of seasonal patterns of social call production revealed that the number of social calls recorded at maternity roost sites showed a linear increase from June to September, whereas, the number of bats emerging decreased sharply from August to September.

Simulations of *P. auritus* social calls were used to investigate behavioural responses to calls away from roost sites using the Autobat. *P. auritus* were clearly much more responsive to simulations of their own species' social calls than to the other stimuli tested. This strongly suggests that the responses to the Autobat represent attempts to interact with the source of the stimulus. Recording with ultrasound and infra-red video was conducted to test the bats' responses to the different types of synthesised call and whether these responses varied seasonally.

A female's approach response to the stimulus may represent an attempt to repel a perceived intruder from her foraging area. Alternatively, if calls were used to coordinate foraging by advertising the location of resources to other females that share the range, a response may represent an attempt to move towards such resources. Experiments showed that females were significantly more likely to respond to a stimulus produced within their core foraging area, than in the peripheral area, or outside their foraging area. On the other hand, while females regularly shared foraging ranges with other females, there was little evidence of co-ordination of movements between simultaneously radio-tracked dyads. It was concluded that responses to the stimuli probably represent attempts to repel perceived intruders from the foraging area.

The thesis concludes with a discussion of some of the advantages and limitations of using play-back of synthesised social calls in the field to investigate vocal communication in bats. Ways in which studies of captive bats of known relatedness could be used to further elucidate the functions of social calls are discussed.

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1.1 - Animal communication

Animal communication can be defined in many ways but, essentially, it involves the transfer of information from one animal to another. An exchange of information can either occur intra-specifically (between individuals of the same species) or inter-specifically (between members of different species). This may, or may not, involve a specific behaviour or signal, and the receiver may, or may not, act upon the information received. Animals use a variety of signal types, which can either be olfactory, visual or auditory in nature to communicate information such as territory ownership (Cooney and Cockburn, 1995; Katti, 2001; Hurst and Beynon, 2004), the presence of predators (Schwagmeyer, 1980; Macedonia, 1990) or to advertise the detection of a food source for species that co-operatively hunt (Bednarz, 1988; Bshary et al., 2006).

1.2 – Vocal communication

Vocal communication, communication produced via the vocal apparatus, is a common communicative signal used by a variety of different animals, for example, birds, anurans, elephants, canids, primates and bats. The production of vocal signals can sometimes have significant associated costs for the individual emitting the signal. For example, the rate at which fringe-lipped bats *Trachops cirrhosus* capture frogs in the Neotropics is significantly higher when the frogs are producing calls to attract females (Tuttle and Ryan, 1981). Male tungara frogs *Physalaemus pustulosus* produce several mating call variants and one call in particular, the 'chuck' call is preferred by females. However, emitting a chuck call makes a male *P. pustulosus* much more vulnerable to predation by *T. cirrhosus* (Ryan et al., 1982).

Not only are there inherent risks associated with behaviour that increases conspicuousness of individuals to predators, it has also been demonstrated that, for some animals, there is an energetic cost in producing the vocal signal. For example, it has been shown that oxygen consumption in the gray treefrog *Hala versicolor* is 21 times greater than consumption at resting (Taigen and Wells, 1985). There are also significant energetic costs in some loud calling mammals, such as red deer *Cervus elaphus* (Cluttonbrock and Albon, 1979; Reby and McComb, 2003). However, the energetic cost of vocal production may be less significant for other animals, such as songbirds (Oberweger and Goller, 2001).

The costs of vocal communication are offset by the benefits (to the individual). These benefits, which are not mutually exclusive, include attracting mates (Catchpole et al., 1984; Buchanan and Catchpole, 1997; Buchanan and Catchpole, 2000), advertising ownership and deterring competitors (Goldberg and Ewald, 1991), broadcasting information on physical quality (Cowlishaw, 1996), broadcasting social information (Leong et al., 2005), broadcasting information about the physical environment (Wrangham, 1977; Hauser and Wrangham, 1987; Wilkinson and Boughman, 1998) and maintaining contact with or recruiting social companions (McComb et al., 1994; Grinnell et al., 1995; Grinnell and McComb, 1996).

1.3 - Communication in microchiropteran bats

Microchiropteran bats produce vocalisations for two purposes: echolocation and communication. For echolocation, pulses of ultrasound are emitted through the mouth or through the nose and returning echoes are interpreted by the bats (Griffin, 1958; Metzner,

1991; Speakman, 2001) which allows them to estimate the distance and location (or the direction of flight) of prey (Neuweiler, 1989) and to navigate through their environment (Holland, 2007; Holland, 2009). Bats also produce communication calls, whose primary function is thought to be communication with other bats, and these are often referred to as social calls

A great deal of research has been carried out on the function of echolocation calls in bats (Jones, 1995; Obrist, 1995; Arita and Fenton, 1997; Schnitzler and Kalko, 2001) and call structure has been described for a variety of species (Fenton and Bell, 1979; Fenton and Bell, 1981; Waters and Jones, 1995; Waters et al., 1995; Vaughan, 1997b; Jones et al., 2000; Russo and Jones, 2002). In contrast, much less is known about the characteristics of the social calls of bats. This may be in part because social calls are given much less frequently than echolocation calls, which are typically produced continuously during flight and, as a consequence, are recorded by researchers less often. Even when social calls are produced frequently in certain situations, they are more specific to particular contexts than echolocation calls. Furthermore, social calls are often emitted in situations where it is difficult to ascertain the social context in which the call was produced (Brown et al., 1983; Kanwal et al., 1994; Andrews et al., 2006; Ma et al., 2006), and it is often not possible to determine which individual is producing the call. Other studies have described the context in which social calls were given (Barclay and Thomas, 1979; Aldridge et al., 1990; Andrews et al., 2006), but have been unable to assign function to call type, due to the inherent difficulties in studying communication in small, fast-flying nocturnal mammals.

1.3.1 - Echolocation

Sound plays a vital role in the lives of Microchiropteran bats, all of which have the ability to echolocate. Echolocation and social calls can be distinguished in terms of their primary function, but there is some overlap. The echolocation calls of any bat have the potential to communicate (intentionally or unintentionally) its identity, location and activity. For example, a study by (Barclay, 1982) on little brown bats *Myotis lucifigus* found that echolocation calls can influence the behaviour of conspecifics and bats of other species. Barclay (1982) used field playbacks of echolocation calls from *M. lucifigus* and attracted conspecifics from up to 50 metres away. *M. lucifigus* was also attracted to playbacks of the echolocation calls of big brown Bats *Eptesicus fuscus* and vice versa. Subadults of *M. lucifigus* responded more to playback of echolocation calls than adults. These results suggest that conspecifics at least, and perhaps other species, may eavesdrop on other bats to locate potential foraging sites (Barclay, 1982).

Furthermore, it has also been demonstrated for a variety of species, including the *E. fuscus* (Kazial et al., 2001; Kazial and Masters, 2004), *M. lucifigus* (Kazial et al., 2008a; Kazial et al., 2008b; Melendez and Feng, 2010), greater mouse-eared bats *Myotis myotis* (Yovel et al., 2009) and the intermediate leaf-nosed bat, *Hipposideros larvatus* (Jiang et al., 2010) that echolocation calls can carry specific signatures that can be used for the recognition of conspecifics and, also, for discriminating between familiar and unfamiliar individuals. For example, playback experiments with lesser bulldog bats, *Noctilio albiventris*, in which individuals were presented with calls of familiar or unfamiliar conspecifics, cohabitant or noncohabitant heterospecifics and ultrasonic white noise as a control, found that bats reacted

with a complex repertoire of social behaviours and the intensity of their response differed significantly between stimulus categories. Stronger reactions were shown towards calls of unfamiliar conspecifics than towards heterospecifics or white noise (Voigt-Heucke et al., 2010).

1.3.2 - Social Calls

Social calls typically differ from echolocation calls in a number of acoustic characteristics. Social calls may be harsh and broadband or tonal and broadband, as described for species such as *M. lucifigus* (Barclay et al., 1979) and are often much longer in duration than echolocation calls, with low frequency components and multiple syllables (Fenton, 1994; Altringham and Fenton, 2003). Although both communication and echolocation calls are of laryngeal origin, there is evidence that separate vocally active brainstem areas are involved in the functional control of communication and echolocation calls (Fenzl and Schuller, 2007). To maximize the distance over which a signal can be heard, communicating bats tend to use lower frequency sounds to minimise the impact of attenuation (Lawrence & Simmons, 1982). For example, the heart-nosed bat *Cardioderma cor* produces short, low intensity echolocation dominated by high frequency sounds (>20kHz), which limits the range over which the calls carry (Vaughan, 1976). The song calls of *C. cor* are much lower in frequency (<20kHz) and therefore are audible over a greater distance. The use of song is seasonal produced when *C. cor* fly about their feeding areas. Feeding success appears to be related to singing at times of low food abundance (Vaughan, 1976), suggesting that it may serve a role in mutual avoidance.

Much of the research into bat vocal communication has focused on vocalisations given in a few specific contexts, such as mating (Barclay and Thomas, 1979; Thomas and Fenton, 1979; Keeley and Keeley, 2004) or during maternal reunion with offspring (Balcombe, 1990; McCracken and Gustin, 1991; Balcombe and McCracken, 1992; Defanis and Jones, 1995; Knornschild and Von Helversen, 2008). Bat also produce social calls at resting places such as roost sites (Chaverri et al., 2010), swarming sites (Parsons et al., 2003) and hibernation sites (Andrews et al., 2006). A number of additional functions proposed for the social calls of bats include:

i. Defence of resources: in areas of high insect density pipistrelle species have been observed foraging in groups of up to 40 individuals without any signs of intra-specific aggression. However, at low insect densities, there was intra-specific aggression (Racey and Swift, 1985). In both *Pipistrellus pipistrellus* and *Pipistrellus pygmaeus* rates of social call production increase as insect density decreases and there is a significant reduction in activity by conspecifics during playback of social calls (Barlow and Jones, 1997). This suggests that food resources are being defended and that social calls have an agonistic function towards other individuals of the same species when insects are scarce.

ii. Group cohesion: Wilkinson and Boughman (1998) found that greater spear-nosed bats *Phyllostomus hastatus* give loud, broad-band 'screech' calls when foraging and these calls appear to function as contact calls that recruit and co-ordinate foraging among group members.

iii. Mobbing: captured *Pipistrellus pipistrellus* emit distress calls that are colony-specific and may attract conspecifics to 'perform mobbing behaviour as an anti-predator response' (Russ et al., 1998).

iv. Attracting mates: (Behr and von Helversen, 2004) found that courtship songs were exclusively observed when male *Saccopteryx bilineata* displayed towards a female *S. bilineata.* In contrast to territorial songs (which were short, rather stereotyped and not obviously directed towards a certain conspecific), courtship songs were long and complex and consisted of highly variable elements ("calls").

v. Territorial male interactions: during the spring, males *Pipistrellus pipistrellus* start setting up individual territories around day roosts. Intruders into the territory are chased away by the resident male, who produces calls that are presumed to be agonistic (Lundberg and Gerell, 1986).

Each of the studies listed above focused on a particular vocalisation type produced in a specific context. However, it is most likely that each species of bat produces a variety of calls that differ in function (Pfalzer and Kusch, 2003). A small number of studies have examined multiple vocalisations within a species and have revealed an extensive repertoire of vocalisations (Barclay et al., 1979; Kanwal et al., 1994; Ma et al., 2006; Melendez et al., 2006) with evidence of syntax (Kanwal et al., 1994; Bohn et al., 2009) and of call composition that varied according to the behavioural contexts in which they were emitted (Bohn et al., 2008). Such variation in vocalisation potentially allows the communication of information concerning the sender and/or situation. If vocalisations are associated with different behavioural contexts, they are likely to have different meanings that should be reflected in their acoustics (Bohn et al., 2008).

1.4 - Approaches to studying communication in animals

One method employed by researchers to investigate the function of vocalisations is to record the sound and play it back to the target species, and examine the response. The use of playback provides a powerful tool for investigating cognitive abilities in animals. Replaying recordings of vocal signals may reveal what information is contained in the call (McGregor, 1992). For example, a study on the female copulation calls of barbary macaques *Macaca sylvanus* revealed that males can discriminate female copulation calls given at different stages of the oestrus cycle. Playback of female calls revealed that males responded more strongly (by looking at or approaching the loudspeaker) around the time when conception was most likely to occur, in comparison to their responses to female calls given around the time the time conception was least likely to occur (by looking away or ignoring the loudspeaker). This suggests that the calls of *M. sylvanus* may contain information on reproductive state, which males can perceive and use in such a way as to increase their reproductive success (Semple and McComb, 2000).

1.5 - Approaches to studying communications in bats

Playback experiments have been used to investigate behavioural responses of bats to a variety of different call types, both echolocation (Barclay, 1982; Voigt-Heucke et al., 2010) and social (Fenton et al., 1976; Barlow and Jones, 1997; Russ et al., 1998; Wilkinson and Boughman, 1998). Playback provides a tool to facilitate the study of bat social calls when it is not practical to study the emission of social calls. However, playback of ultrasound is problematic in that the equipment required for recording and reproducing ultrasound frequencies is expensive and fragile for regular use in the field (Hill and Greenaway, 2005). Furthermore, it can be difficult to obtain good quality recordings of social calls, because they

are given infrequently, and there is often background noise. In order to address this problem Hill and Greenaway (2005) developed an acoustic lure, in the form of a portable ultrasound synthesiser, the Sussex 'Autobat', which can be programmed to emit simulated bat social calls. The lure was initially developed to attract elusive woodland bats, such as Bechstein's bat *Myotis bechsteinii*, and field studies demonstrated that broadcasting simulated social calls at woodland sites attracted bats to mist nets and significantly increased captures rates (Hill and Greenaway, 2005; Hill and Greenaway, 2008).

Preliminary fieldwork at a variety of woodland sites showed that bats responded well to the Autobat, and that brown long-eared bats *Plecotus auritus* responded particularly well to simulated social calls. The Autobat output is modelled on bat social calls, but the repetition rates are much higher. So one of the central questions was 'why do bats respond'? Also, what, if anything, can this tell us about the function of social calls? It would be totally impractical to investigate the function of social calls by playing them at anything approximating natural rates, because these are so low, that it would be difficult or impossible to obtain sufficient results. Preliminary fieldwork also suggested that bats were attracted to a range of stimuli, modelled on social calls, so an alternative was to investigate the bats' responses to these, whether responses to different stimuli differed, and whether responses varied in relation to spatial and seasonal context.

1.6 - The study species brown long-eared bat *Plecotus auritus*

1.6.1 - Distribution

The brown long-eared bat (*Plecotus auritus*, Linnaeus 1758) is a medium-sized Palearctic vespertilionid with a widespread distribution. *P. auritus* range in Europe extends westward to the west of Ireland (Shiel et al., 1991), as far south as central Spain (Fernandez, 1989) and central Italy (Crucitti, 1989), and as far north to Sweden (where the northern edge of the range appears to be 63^0N) (Ahlen and Gerell, 1989). It was also thought that *P. auritus* extended eastwards as far as Sakhalin and Japan (Corbet and Hill, 1991) and in Asia it was found in Mongolia and north-east China (Zheng and Wang, 1989). However, a recent study using a combination of morphological and molecular data revised the genus and concluded that *P. auritus* is restricted to Europe including the Ural and Caucasus mountains (Spitzenberger et al., 2006). *P. auritus* is common and widespread in Britain and is distributed throughout the country except in the far north and north-west of Scotland and offshore islands (Swift, 1991). It has been recorded in a variety of habitat types but is usually associated with woodland or parkland.

Two species from the genus *Plecotus* occur in Britain; the brown long-eared bat *P. auritus* (Linnaeus 1758) and the grey long-eared bat (*P. austriacus*, Fischer 1829). The two species are morphologically very similar but can be distinguished through a set of measurements used in combination (Stebbings, 1967; Greenaway and Hutson, 1990; Swift, 1998). The two species occur sympatrically in central Europe, but *P. austriacus* is generally found at lower altitudes and latitudes than *P. auritus* (Swift, 1991; Altringham, 2003). In Britain *P. austriacus* is at the northern edge of its distribution. It is one of the rarest British bat species,

and only found along a thin strip of southern England from the coastal areas of Devon to West Sussex (Altringham, 2003). They are more common on the Isle of Wight and the Channel islands.

1.6.2 - Morphology

P. auritus weighs approximately 6 – 12 grams in weight, with a wing span of 230 – 285mm, forearm length of 34 – 42mm and a body length of 37 – 48mm. Species of the genus *Plecotus* are characterised by their long ears, with pinnae length of 29-38mm, which is well over half that of the body (Swift, 1991; Swift, 1998). The wings are short and broad with a low aspect ratio and low wing loading (Norberg and Rayner, 1987). The wing shape of *P. auritus* allows for slow, fluttering, manoeuvrable flight in cluttered habitats (such as woodland) (Aldridge and Rautenbach, 1987; Sevcik, 2003). It frequently hovers (Entwistle et al., 1996) and can rise vertically for a few feet (Norberg, 1976) and this enables the species to forage by gleaning (capturing prey that is crawling or at rest on solid surfaces rather than in flight). The large ears can pick up return echoes from short low intensity calls (Waters and Jones, 1995) and this species also uses passive listening of prey–generated sounds to locate prey (Anderson and Racey, 1991; Anderson and Racey, 1993). Laboratory experiments have also shown that *P. auritus* use visual cues for detection, but exploit additional information, such as echolocation and passive listening, during the final pursuit (Eklöf and Jones, 2003).

1.6.3 - Annual cycle of behaviour and reproduction

Plcotus auritus displays a seasonal cycle of behaviour in common with other temperate bat species. Colonies form in spring and last throughout summer, with autumn and winter mating, sperm storage and delayed fertilisation (Swift, 1998). When *P. auritus* emerges from hibernation in the spring, the bats spend time in transient roosts before the females come together to form maternity colonies in the late spring early summer. However, *P. auritus*, is unusual among temperate zone bats in that summer maternity colonies include adult males as well as females (Speakman et al., 1991; Entwistle et al., 2000; Burland et al., 2001). Estimates of colony size vary with some researchers on the continent estimating as few as 5 – 10 on average (Horáček, 1975), to estimates of over 80 females for some colonies (Swift and Racey, 1983; Battersby, 1999).

Sexual maturity begins for a few individuals (5 – 10%) in the first autumn but, for most *P. auritus* sexual maturity is attained in their second year (Speakman and Racey, 1986; Entwistle et al., 1998). The first mating can be observed in mating roosts in August and the last in spring gathering roosts in April (Furmankiewicz, 2002; Furmankiewicz, 2008). The gestation period is between 60 and 70 days (Swift, 1981; Speakman and Racey, 1987) and a single young is produced in June or July (Swift, 1981). The juveniles become volant at around 30 days and are usually fully weaned at six weeks (Swift, 1981). Hibernation begins from around the end of October through until April but bats can emerge to feed and drink throughout the winter (Hays et al., 1992).

1.6.4 - Roosts

In parts of central Europe, *P. auritus* are known to roost in tree cavities (Horacek, 1975), and it seems likely that these are the natural roost sites for the species (Swift, 1998; Dietz et al., 2009). In Britain, summer maternity colonies are most frequently found in buildings (Stebbings, 1966; Jones et al., 1996; Entwistle et al., 1997; Battersby, 1999), but bat boxes (Boyd and Stebbings, 1989) and tree roosts (Murphy *et al.* in prep) are also commonly used, particularly in southern Britain. Temperature has been shown to be an important factor in summer roost selection (Entwistle et al., 1997; Battersby, 1999).

Plecotus auritus hibernates in a variety of structures including buildings, underground sites such as caves, well pits and railway tunnels and, also, in tree holes (Swift, 1991). *P. auritus* is a non-migratory species with the longest recorded movement being 90km (a female from Germany noted in Dietz et al., 2009).

1.6.5 - Foraging

The wing and ear morphology, coupled with low intensity echolocation, suggest that *P. auritus* is adapted to feeding in cluttered habitats (Norberg and Rayner, 1987) and studies have shown that the species feeds predominately in woodland (Entwistle et al., 1996). *Plecotus auritus* emerge from their day roosts fairly late relative to many other verspertilionid species and this late emergence is almost certainly connected with their habit of gleaning (Rydell et al., 1996; Swift, 1998). The time of emergence in insectivorous bats is likely to be a compromise between the need to emerge to feed and avoiding the risk of predation at high light levels (Speakman, 1991; Jones and Rydell, 1994). *Plecotus auritus* usually use

landscape features, such as hedges or tree-lines, to fly between day roosts and foraging sites and tend to avoid flying in the open (Entwistle et al., 1996). This may reduce the risk of predation by nocturnal birds, such as tawny owls (Speakman, 1991; Lesinski et al., 2009a; Lesinski et al., 2009b), barn owls (Speakman, 1991; Petrzelkova and Zukal, 2003) and birds of prey, such as hawks or raptors, flying at dusk (Speakman, 1991; Fenton et al., 1994). *Plecotus auritus* has been described as a woodland bat and occurs in a variety of woodland types including deciduous (Entwistle et al., 1996), coniferous (Fuhrmann and Seitz, 1992) through to birch scrub and gardens with mature trees (Swift and Racey, 1983). It has also been recorded in orchards and parkland among meadows (Barataud, 1990). Research in Germany and Sweden recorded frequent use of conifer forest (Fuhrmann and Seitz, 1992; Ekman and DeJong, 1996), while in north-east Scotland the species showed a significant preference for deciduous, broadleaved woodland, and utilised only the edges of conifer plantations (Entwistle et al. 1996).

1.6.6 – Diet

Early studies based on insect remains found under feeding perches suggested that *P. auritus* was a moth specialist and consumed little else (Poulton, 1929; Buckhurst, 1930). However, more recent investigations, based predominately on faecal analysis, have shown that the species is flexible in its diet and takes a variety of prey including non-flying arthropods such as Araneae and Dermaptera (Swift and Racey, 1983; Rydell, 1989; Shiel et al., 1991; Vaughan, 1997a). The remainder of the diet consists of moths, Coleoptera and Diptera (Vaughan, 1997a). Prey items may also be caught inside the roost, both in summer roosts (Swift and Racey, 1983; Rydell, 1989) and winter hibernation sites (Roer, 1969). Feeding perches are often used to consume larger prey items (Thompson, 1982; Robinson, 1990).

1.6.7 - Conservation status

Plecotus auritus is one of the more common bat species in the British Isles and parts of Central Europe, with a population estimate of 245,000 individuals in Britain (Battersby, 2005). The 2009 IUCN Red List (IUCN, 2009) classifies the species as "Least Concern" and the population trend as "Stable". However, the National Bat Monitoring Programme reported an estimated 20% decrease for this species in the UK over a period of seven years (BCT, 2004), which led to its inclusion in the revised List of Priority Species for the UK Biodiversity Action Plan (B.R.I.G., 2007).

1.6.8. - Echolocation and social calls

P. auritus is sometimes described as 'the whispering bats' as their echolocation calls are characterised by their quietness with a fast pulse rate (Russ, 1999). The call is a short FM sweep, approximately 2ms in duration, with a fast pulse rate that can be heard most clearly on a detector set between 30kHz and 40kHz, but it is very faint and only detectable if the bat is less than about five metres away (Swift, 1998).

Ahlén (1981) identified a loud call detectable at 40 metres or more, that was longer in duration (7.1ms) and descended in frequency from about 42kHz to about 12kHz, ending with a short (1ms) CF portion at about 12kHz, which was also the frequency of maximum energy followed by a short downward FM sweep (Ahlen, 1981). Ahlén (1981) noted that this call type was emitted intermittently when *P. auritus* was flying inside barns and mines and more regularly when flying in the open. Coles *et al.* (1989) demonstrated that this peak frequency of 12kHz coincided with the centre of the most sensitive range of *P. auritus* hearing and

proposed that this call could be a long, distance communication call (Coles et al., 1989). However, the function of this call was not determined.

Simulation of *P. auritus* social calls using the acoustic lure represents a novel approach for investigating the function those calls. For example, if *P. auritus* social calls away from roost sites are used to defend resources, then it would be expected that *P. auritus* females would respond more frequently to calls broadcast within their foraging range compared to calls broadcast outside of their foraging rage. Alternatively, it could be that social calls away from the roost sites are used in long distance communication to co-ordinate foraging behaviour, then it would predicated that there would be evidence of co-ordinated foraging behaviour amongst colony members.

P. auritus regularly emit social calls at summer roost sites around the time of entering and exiting the roost (Entwistle, 1994; Battersby, 1999) and during dawn swarming at roost sites. It is likely that some of the calls emitted at roosts sites will be in the context of maternal care. Isolation calls (i-calls) emitted by infant *P. auritus* have been shown to have individual variation, and laboratory experiments demonstrated that vocal signatures allowed adult females to recognise and suckle their own pup (Defanis and Jones, 1995). However, *P. auritus* also emit also social calls at roost sites during gestation, which cannot be related to maternal care. Alternative hypotheses would be that these calls are related to processes such as group cohesion or information transfer. If social calls at roost sites were a function of group cohesion, then it would be expected that the number of calls would be positively correlated with the number of bats in the roost and there would be evidence of variation in social call structure at roost sites.

Furmankiewicz (2004) reported that *P. auritus* also emit social calls at swarming sites in Continental Europe in spring (March-April) and in autumn (September-October) and speculated that these calls could function as mating calls. However, the frequency of occurrence of *P. auritus* at swarming sites in the UK seems to be less than on the continent (Furmankiewicz, 2002; Parsons et al., 2003; Veith et al., 2004; Furmankiewicz and Altringham, 2007) and alterative mating sites (such as roosting locations near to or within disbanded maternity colonies), may provide male bats with more regular access to females. If social calls at roost sites were related to mating, then it would be expected that the number and variation of calls would increase in the mating season.

1.7 - The study area

The study was conducted at various woodland, village and farm sites across the counties of East and West Sussex, southern England. Sussex is one of the most wooded counties in Britain with about 15% of the county covered by woodland (Oakes and Whitbread, 1988). Sussex is also one of the warmest counties in Britain with a mean minimum temperature of approximately 3^{o}C, and mean maximum temperature of approximately 20^{o}C. Mean annual rainfall ranges from 800mm along the coast to 1200mm on higher ground (MetOffice, 2010). Sussex is a relatively densely populated county with a population estimate of 1.38 million people at the last 2001 census. This represents an estimated population rise of 7% since the census of 1991 (National Statistics Office, 2010) and many rural towns have expanded, reducing the amount of semi-natural habitat available in the county.

1.8 - Specific aims and objectives

The overall aim of my research was to examine the nature of social calls of brown long-eared bats *Plecotus auritus* and improve and expand our knowledge of their functions and characteristics. *P. auritus* produce social calls frequently at roosting sites and much less frequently when foraging. This thesis will examine the nature of social calls recorded at roost sites and responses to them in the field through a combination of recording and playback experiments. At the same time the study will examine aspects of the behavioural ecology of the species, specifically relating to ranging behaviour, nocturnal activity patterns and patterns of maternity colony use.

Specific objectives of my study were as follows:

1. To describe the structure and variability of *P. auritus* social calls in terms of their acoustic parameters;

2. To examine temporal patterns of social call production at summer roosting sites;

3. To describe behavioural responses of *P. auritus* to simulated social calls;

4. To assess the extent to which sex and season influence the responses of *P. auritus* to simulated social calls;

5. To determine whether responsiveness to simulated social calls varies according to the location of the stimulus in relation to a female's foraging area; and

6. To assess the extent to which females occupying adjacent or overlapping ranges coordinate their ranging movements.

In order to address these objectives I present, in Chapter 2, an analysis and classification of the social calls of *P. auritus* recorded at 20 maternity roost sites across three geographic locations. In Chapter 3 I go on to describe the patterns of social call production at the 20 maternity roost sites, in relation to the size of the colony and, also, the time of day and season. In Chapter 4 I assess the difference in behavioural responses to a variety of stimuli, and how responses to simulations of *P. auritus* social calls vary seasonally. Chapter 5 describes a pilot study used to develop a methodology for a radio-tracking experiment, to examine responses of *P. auritus* and the subsequent radio-tracking study to establish home range use by females. Chapter 6 details a preliminary study to determine reliable distances at which *P. auritus* respond to social calls and the trialling of a system to indicate the approach of a radio-tagged individual. I assess whether the propensity of *P. auritus* to respond to stimulus calls is dependent on the stimulus location in relation to foraging area. Patterns of range use and interactions between simultaneously radio-tracked pairs of female *P. auritus* are investigated in Chapter 7. The thesis concludes with a general discussion in Chapter 8.

2.1 - Introduction

Classification of the kinds of signals animal use is an important step towards understanding their communication. Classification of vocal signals has been carried out fairly extensively for a variety of different species of birds (Weary 1990, Armstrong 1992, Horning et al., 1993; Burnell and Rothstein, 1994; Ficken et al., 1994; Nowicki et al., 1994; Gentner and Hulse, 2000; Baker and Logue 2003; Ranjard and Ross, 2008), cetaceans (McCowan, 1995; Janik, 1999; Rendell and Whitehead 2003; Boisseau, 2005; Shulezhko and Burkanov, 2008; Soldevilla et al., 2008), and primates (Mitani and Marler 1989; Ambrose 2003; Becker et al., 2003; Rukstalis et al., 2003; Miller and Hauser, 2004; Savigh et al., 2007; Gamba and Giacoma, 2007; Chakladar et al., 2008; Pozzi et al., 2010). Studying the vocal communication of bats presents a particular challenge, compared to most birds and other diurnal animals, because of their nocturnal habits, often inaccessible roosts and the ability to fly. These factors are likely to have hindered the study of communication in bats in their natural habitat.

A number of studies have described the signal structure of various types of communication calls in a given context. Examples include infant isolation calls (Gelfand and McCracken, 1986; Defanis and Jones, 1995; Knornschild and von Helversen, 2008), mating calls (Barclay and Thomas, 1979; von Helversen and von Helversen, 1994; Behr and von Helversen, 2004), and group contact calls whilst foraging (Wilkinson and Boughman, 1998). A few studies have investigated multiple vocalisations within a species. These studies have shown that for some species of bats communication calls may consist of several variations and combinations of CF and FM signals (Kanwal et al., 1994; Pfalzer and Kusch, 2003; Andrews et al., 2006; Ma et al., 2006; Knornschild et al., 2010) and may include evidence of syntax (patterns in the

sequences of calls given) (Kanwal et al., 1994; Bohn et al., 2008). Furthermore, research has shown that some species of bat are capable of vocal learning (Esser, 1994; Boughman, 1998).

Many studies investigating bat social call structure have described individual elements of the 'calls' through visual examination of sonograms (Fenton, 1976; Barclay et al., 1979; Barclay and Thomas, 1979; Pfalzer and Kusch, 2003; Furmankiewicz, 2004; Jahelkova et al., 2008). The acoustic parameters of a call may be important for conveying information, but there are other features of vocalisations that may also be of importance, i.e. the temporal distribution of call emission, whether calls are emitted singly or in distinct clusters (Kanwal et al., 1994; Bohn et al., 2008), how frequently calls are emitted over time (Hauser, 1998), and whether calls are emitted as sequences (Bohn et al., 2009). Furthermore, visual inspection of calls with the aim of classifying them into separate groups, based on spectrogram structure alone, is generally qualitative, difficult to conduct on large datasets and, therefore, subject to the individual interpretation of the researchers (Melendez et al., 2006).

To understand the acoustic communication of a species it is essential to distinguish between different call types. As animal communication is likely to be influenced by the context in which it occurs, it is useful, in as a first step to focus on a single context. This chapter presents the results of acoustic analysis of *P. auritus* social calls produced in the vicinity of a number of maternity roosts is Sussex. Calls were recorded continuously through the night, and in each of the five summer months. The aims of this chapter are to describe the structure of social calls recorded at roost sites and to employ statistical methods to objectively classify the calls.

2.2 - Methods

2.2.1 - Recording calls

An automated ultrasound recorder was placed at the main exit location for emerging *P. auritus* at 20 maternity roost sites. All ultrasound was recorded from dusk to dawn, on a monthly basis from May through to September, at each of the 20 locations (see Chapter 3 for more detailed roost information and patterns of social call production). Automated remote ultrasound detectors allow large amounts of data on bat presence and vocal activity to be collected. This study used a custom-built ultrasound monitoring device, called the Ultrabat, shown in Figure 2.1, that records data directly onto a hard disk. Once the Ultrabat has been triggered by a bat call, or other ultrasound, it records for a preset period of three seconds. Once the three second sampling period has finished, there is a 10ms interval delay to allow the record to be written to the file. The file is then stored on the hard disc along with the time and date that the file was created. The Ultrabat produces high quality recordings that are suitable for quantitative analysis using sound analysis software. The microphone within the Ultrabat system is a Knowles EK2132 model. This broadband microphone is used commonly by many standard commercial bat detector models (such as Petterson time expansion detectors & Wildlife acoustics SM2). However, the frequency response of this microphone was measured (see Appendix A.4.3.2) and it has low frequency roll-off and does not record frequences below about 10kHz, which means that parameters of the call below this frequency are not captured accurately. Unfortunately, social calls do seem to have low frequency components that my recordings did not capture.

2.2.2 - Analysis of calls

The most common techniques currently available to convert the ultrasonic vocalisations of bats into audible sound include frequency division, heterodyne, and time expansion (for reviews see of Parsons et al., 2000; Parsons and Obrist, 2004. The Ultrabat records ultrasound directly and a custom-designed conversion program (datTOwav) converts the binary data files to wav files. These wav files were analysed in time expansion on a personal computer (Dell Inspiron 2200 using the software Batsound (v3.31 Pettersson Elektronik AB, Uppsala) with a sampling frequency of 31270Hz, 16 bits/ sample, and a 512pt. FFT with a Hamming window for analysis.

For each social call recorded, the following parameters were measured: starting frequency (Fstart), the frequency value measured at the beginning of the call; maximum frequency (Fmax), the highest frequency value observed within the call; end frequency (Fend), the frequency value measured at the end of the call; minimum frequency (Fmin), the lowest frequency value observed within the call; frequency of maximum energy (Fmaxe); total call duration (Dur); and inter-call interval (ICI, the time interval between two consecutive social calls). Figure 2.2 shows a sonogram of two typical *P. auritus* social calls with the various call parameters highlighted. ICI and D were measured from oscillograms, Fmaxe (kHz) from power spectra, and all other spectral parameters (kHz) from spectrograms. The time between consecutive social calls was measured in milliseconds. If the time between social calls was longer than two minutes, then the time was estimated to the nearest second. If more than one harmonic was present, values of the first (fundamental) harmonic were taken. Incomplete records and those with poor resolution were excluded from the analysis.

Calls that were clearly identified as *P. auritus* echolocation calls (as shown in Figure 2.3) were excluded from the analysis. *P. auritus* are sometimes referred to as whispering bats due to the quietness of their echolocation calls, which are emitted through their mouth or nostrils (Schober and Grimmberger, 1997). The echolocation call of *P. auritus* was analysed by Ahlén (1981) who described it as a faint and short FM sweep, about 2 ms long with prominent second harmonics, and a fast pulse rate (Russ, 1999).

2.2.3 – Principal Component Analysis

Patterns in data can be hard to find in multi-dimensional and large datasets (such as the call database described in this chapter). Principal Component Analysis (PCA) is a powerful tool for identification of patterns in data and to present the data in such a way as to highlight the similarities and differences. PCA begins with a matrix that represents the relationship between variables. The mathematical technique used in PCA is called eigen analysis and the eigenvalues and eigenvectors of the matrix are calculated using the sums of squares and cross products (Jolliffe, 1986). The eigenvector associated with the largest eigenvalue has the same direction as the first principal component. The eigenvector associated with the second largest eigenvalue determines the direction of the second principal component. The sum of the eigenvalues equals the trace of the square matrix and the maximum number of eigenvectors equals the number of rows (or columns) of the matrix (Jolliffe, 1986). Therefore, the first principal component is the linear combination that explains the most variance and the last principal component is the remaining combination of features that explains the least variance in the data (Wold et al., 1987).

The six acoustic parameters (Duration, Fmin, Fmax, Fstart, Fend, Fmaxe) were subjected to a PCA in order to explore the variance within the dataset. Not all components are retained in the analysis, and there is debate over the criteria used to decide whether a factor is statistically important. Only components with large eigenvalues should be retained, but deciding what constitutes an eigenvalue large enough to represent a meaningful component is more problematic. One method devised to decide the number of components is to plot a graph of each eigenvalue against the component with which it is associated. This graph is known as a scree plot and the cut off point for selecting components should be at the point of inflexion of the curve (Cattell, 1966). However, Kaiser (1960) recommended retaining all components with eigenvalues greater than one. This is based on the premise that eigenvalues represent the amount of variation explained by a principal component and that an eigenvalue of one represents a considerable amount of variation (Kaiser, 1960). With these considerations in mind, a scree plot was constructed to help establish the number of components to be retained, in addition to Kaiser's (1960) recommendation of retaining only principal components with an eigenvalue greater than one.

2.2.4 – Cluster Analysis

The objective of cluster analysis is to separate a set of objects into constituent groups (classes, clumps, clusters) so that members of any one group share similar properties and differ from one another as little as possible, according to a chosen criterion (Späth, 1980). The ultimate objective is to minimise within-group variance and maximise between-group variance. Almost all clustering techniques involve a process of measurement, either of the magnitude of distance between two objects, or of the magnitude of their similarity to each other, where objects are described by the values of the variables in the data matrix. The

choice of measurement used will influence the shape of the clusters, as some elements may be close to one another according to one distance and farther away according to another (Romesburg, 2004). The most straightforward and most common technique of calculating distances between clusters in a multi-dimensional space is to calculate Euclidean distances. However, the Euclidean distances can be greatly affected by differences in scale among the dimensions from which the distances are calculated and, therefore, transforming the dimensions to ensure that there are similar scales is an important step.

Once the distances between objects have been determined, the next step in the clustering process is to create the clusters. There are a number of different linkage and amalgamation rules that exist to help determine when two clusters are sufficiently similar to be linked together. For example, single linkage determines the distances between two clusters by the distance of the two closest objects (i.e., by the nearest neighbours) whereas complete linkage determines the distance between clusters by the greatest distance between any two objects in the different clusters (i.e., by the furthest neighbours). The choice of linkage or amalgamation rule depends in part on whether the objects actually form naturally distinct clumps or whether the objects are elongated 'chain like' clusters (Hartigan, 1975).

Traditional clustering methods fall into two broad categories: partitioning and hierarchical. Partitioning methods (such as k-means) move records iteratively from one cluster to another, starting from an initial partition. However, the number of clusters must be specified in advance, which is arbitrary, and this does not change during the iteration. Furthermore, k-means, for example, does not handle outliers very well (Jain and Dubes, 1988). Hierarchical clustering is advantageous in that, unlike partition methods, you don't need to specify the number of clusters in advance. However, it is difficult to process with large datasets, as it is

slow and problematic when the data contains a high level of error. Therefore, both of these traditional clustering methods were considered unsuitable for the initial cluster analysis because (i) the number of the clusters could not be identified objectively and (ii) the database of social calls was very large. More recently probability models have been proposed (Banfield and Raftery, 1993; Dasgupta and Raftery, 1998; Fraley and Raftery, 1998) using the Bayesian information criterion to compare multiple models and identify the optimum number of clusters. The model based cluster analysis approach has been shown to perform statistically better than the traditional methods (Fraley and Raftery, 1998) and also provides a measure of the uncertainty of the resulting classification.

With these considerations taken into account, cluster analysis using the model-based approach (Fraley and Raftery, 1998) was conducted on the call database (data matrix) for whereby the calls are classified on the values of the variables, which are the measured acoustic parameters (Duration, Fmin, Fmax, Fstart, Fend, Fmaxe). The resulting factor scores from the PCA (with an eigenvalue greater than one) were also subjected to a cluster analysis. The clustering technique used was MCLUST extension to R statistical software (Fraley and Raftery, 2006). The cluster model and number of clusters were further verified by subjecting a random selection of 75% of call database to MCLUST a further five times, each time using a random selection. As an additional verification, a supervised clustering was also conducted, using a traditional partitioning method, k-means cluster analysis, whereby the analysis specified to split the calls into the number of clusters determined by the model based approach. This was then compared to the model based analysis in order to ascertain whether there was agreement between both methodologies.

2.2.5 – Analysis of call sequences using Markov chain analysis

In order to examine whether there was any evidence of sequences of calls, in the form of syntax (patterns in which call types from the resulting clusters are ordered and combined) a first order Markovian chain analysis was applied. A Markov chain is a process that consists of a finite number of states and some known probabilities p_{ij}, where p_{ij} is the probability of moving from state *j* to state *i* (Norris, 1998). The controlling factor in a Markov chain is the transition probability; it is a conditional probability for the system to go to a particular new state, given the current state of the system (Revuz, 1984). The computer programme generated a matrix of the probability that one call type immediately followed the same or a second call type for each call type in the data set.

2.2.6 - Statistical Analysis

The descriptive analysis (mean, standard deviation, standard error, variance) and K-means clustering was carried out using SPSS (version 17.0 for Windows) for all of the 11,464 call parameters measured. A custom-designed MATLAB program, written by Lucas Wilkins at the University of Sussex, performed the PCA and MCCLUST Version 3 for *R* (Fraley and Raftery, 2006) carried out the model based cluster analysis. The ellipses for the clusters were drawn using a custom designed programme in Phyton v2.6 for Windows. The analysis of call sequences (Markov chains) were conducted using a custom designed programme in Phython v2.6 for Windows.

2.3 - Results

A total of 11,484 *P. auritus* social calls were recorded at the 20 maternity roost sites from May 2007 to September 2007.

2.3.1 - Descriptive variability of P. auritus *social calls at maternity roost sites.*

Analysis of ultrasound social calls (n= 11,484) showed that the majority of calls (n = 11,101) were comprised of a downward FM sweep, descending in frequency from a mean of approximately 52kHz to about 14kHz. A sonogram of a typical *P. auritus* 'Type A' call is shown in Figure 2.4. There was, however, a lot of variation within the acoustic parameters measured, detailed in Table 2.1 and Figure 2.5, specifically variations in Fmax(e).

The second most frequent call type (n = 215) recorded, which differed in spectrogram structure to Type A calls, was characterised by an upward sweep followed immediately by a falling FM sweep, as shown in Figure 2.6. This type of call was also described for *P. auritus* recorded at swarming sites by Furmankiewicz (2004). These calls, termed 'Type B', could be differentiated objectively from Type A calls on the basis of their starting frequency being lower than the maximum frequency within the call, as shown in Figure 2.7. The variations within the acoustic parameters measured are detailed in Table 2.2 and Figure 2.8

The final call type (n = 168) that could be distinguished objectively based on call structure, was characterised by a downward FM sweep followed by an undulating FM sweep, as shown in Figure 2.9. This type of call was also recorded by Furmankiewicz (2004) at *P. auritus* swarming sites and was described as 'v calls'. These calls, termed here 'Type C', could be differentiated from Type A and Type B calls on the basis of their end frequency being higher

than the minimum frequency within the call, as shown in Figure 2.10. The variations within the acoustic parameters measured are detailed in Table 2.3 and Figure 2.11.

2.3.2 - Relationship between acoustic parameters of the calls

The relationships between the acoustic parameters of each call were analysed and the results of the Spearman's rank correlation are detailed in Table 2.4. There was a significant negative correlation between the duration of a *P. auritus* social call and both the minimum and end frequency of the call, as shown in Figure 2.12 (a) and Figure 2.12 (b), respectively. Calls that were longer in duration tended to have lower minimum and end frequencies than shorter duration calls. There was also a significant negative correlation between the duration of a *P. auritus* social call and the peak frequency of maximum energy, as shown in Figure 2.12(c). The minimum frequency was positively correlated with peak frequency of maximum energy, as shown in Figure 2.12(d).

2.3.3 – Principal Component Analysis

The results of the PCA analysis, shown in Table 2.5, show that the first principal component accounted for 51.49% of the variance in the data matrix and the second principal component accounted for 31.82% of the variance in the data matrix. The scree plot, shown in Figure 2.13, plots the eigenvalues of the components in descending magnitude. The point of inflexion on the curve showed that there was no single clear elbow. However, only two components had eigenvalues greater than one, therefore, only these were retained.

A 2D loading plot was constructed, shown in Figure 2.14, to examine how the variables load onto each factor. Fmax and Fstart are heavily loaded on the first axis. This is unsurprising as these high frequency call parameters are likely to be extremely variable as a result of the attenuation of calls at high frequencies. The second component is more interesting, with three large positive loadings, Fmin, Fend and Fmax(e) and one large negative loading, duration. This further demonstrates the relationship between call duration and frequencies (described in Section 2.3.2), whereby longer social calls are characterised by low end and minimum frequencies and low frequency of maximum energy. A 3D histogram of the first two components was plotted against frequency. The resulting histogram, shown in Figure 2.15, demonstrates that the social calls tend to overlap in the parameters measured, but that there is some evidence of clusters of calls that have similar distributions in acoustic parameters.

2.3.4 – Model-based cluster analysis

The majority of calls (96.66%) were classified as 'Type A' calls, on the basis of sonogram structure. Although Type A vocalisations shared the same basic pattern, it was a very large group within which there was a lot of variation in acoustic parameters. In order to examine whether Type A calls could be further separated on the basis of the measurements of their acoustic parameters, the call database of Type A calls was initially subjected to a cluster analysis using the acoustic variables measured. The default settings for the EMclust command in MCLUST tested from 1 to 20 clusters and 10 models. The models tested are described further in Fraley and Raftery (2006). The resulting Bayesian information criterion (BIC) was used to determine which was the best model and number of clusters. This initial exploratory analysis revealed that the best model was 'EEV', a model with an ellipsoidal distribution, equal volume, equal shape, and variable orientation, as shown in Figure 2.16.

However, the model appears to over fit the data (which may be as a result of random error or noise) reaching asymptote initially at six clusters but increasing again at nine and 16 clusters. There are also missing values, and this suggests that one or more of the estimated covariance matrices are judged to be too close to singularity (Fraley and Raftery, 1998).

The factor scores from the PCA, with an eigenvalue greater than one, were subsequently subjected to the cluster analysis in MCLUST (as described above) in order to ascertain whether reducing the number of factors resulted in improving the fit of the statistical model. The resulting BIC, shown in Figure 2.17, indicated that the model reached asymptote at four (or arguably five) clusters but began to decrease after six clusters. Subjecting five random selections of 75% of the acoustic parameter data revealed that the EEV model was consistently the best model to fit the dataset, but the number of clusters as having the highest BIC value did change, as shown in Table 2.6. Six clusters, however, did end up in the top three BIC values on all five runs (whereas four and five did not). Therefore, the EEV model with six clusters was chosen as the most conclusive option to describe the call distributions of the data set.

The ellipses representing the six Gaussian clusters fitted to the data distribution are shown in Figure 2.18. The measurements of the acoustic parameters from the call, with the greatest probability of belonging to each of the cluster, are detailed in Table 2.7. Calls in overlapping clusters one and six were characterised by a duration of 9.8 ms and 11.1ms, a minimum frequency of 10.6 kHz and 13.2 kHz, and a frequency of maximum energy of 21.8 kHz and 19.6 kHz respectively. Calls in cluster two were longer in duration (12.4 ms), had similar minimum frequency to cluster one (10.6 kHz), and a lower frequency of maximum energy (14.5 kHz). Calls in clusters three and four were characterised by having a duration of 9.0 ms

and 10.9 ms, a higher minimum frequency (15.0 kHz and 17.3 kHz), and higher frequency of maximum energy (27.2 kHz and 31.2 kHz), but differed from one another in their starting frequencies, which were in the region of 43.7 kHz for cluster three and 52.5 kHz for cluster four. Calls in cluster five were characterised by having duration of 12.3 ms, a minimum frequency of 13.2 kHz, and a frequency of maximum energy at about 19.60 kHz. The clusters were not discrete and overlapped in their distributions. Therefore, there was the potential for some calls to be misclassified into the incorrect cluster. The probability values for the classification of each cluster with another cluster type are shown in Table 2.8.

2.3.5 – K-means cluster analysis

The acoustic parameters of the 11101 Type A calls were used as input into a k-means cluster analysis to determine homogeneous groups. Based on the Bayesian information criterion (section 2.3.5) the analysis aimed to separate the calls into six clusters where each of the calls was allocated to the cluster with the nearest mean and the results are shown in Table 2.9. The Euclidean distances between the final cluster centres are detailed in Table 2.10. The analysis showed that there was, in general, good agreement between the two clustering methods. Clusters two, three and five had a similar number of calls, with similar acoustic properties for both clustering methods. Cluster one differed between the two methods primarly in the acoustic parameter of the minimum frequency which was 10.6 kHz for the model based analysis and 13.4 kHz for the k-means analysis. There were also more calls in cluster one using the k-means (n = 2590) compared to the model based analysis (n = 2084). Cluster four has less calls using the k-means approach (n = 1525) compared to the model based approach (n = 2411) and were shorter in duration (8.72 ms and 10.9 ms respectively). Cluster six had

more calls using the k-means appraoch (n = 2566) compared to the model based appraoch (n = 1972) but, in general, the calls had similar acoustic properties using both methods.

2.3.6 – Sequence analysis – Markov chain

Only the calls from Type A were included in the Markov chain analysis (due to the low frequency of occurrence of Type B and Type C). The probabilities generated from the Markovian chain analysis, shown in Table 2.11, give a preliminary indication of the sequential predictability of call types. As shown in Figure 2.19, calls in cluster one, for example, has a probability of 48% for a call in cluster one to occur immediately after. This is then followed by a call typical of cluster six (19%), a call typical of cluster two (12%) and a call typical of cluster four (11%). Calls typical of cluster three and five have a 1.3% and 6.3% chance of occurring immediately after a call typical of cluster one respectively.

Each of the six call types classified was more likely to be followed by a repeat of the same call type than by any other call type, as shown in Figure 2.19. Call type 1 was first in the sequence (i.e. followed a period of silence) more often than expected and call Type 6 was first less in the sequence more often than would be expected from their frequencies alone, χ^2 1123.85, d.f. 5, $p < 0.0001$; shown in Figure 2.20. The probabilities of a third call type occurring in a sequence, given that the other two have occurred was not analysed. However, it was considered likely that multiple bat vocalisations may have contributed to the sequences which may confound the results.

2.4 - Discussion

A fundamental step for understanding animal auditory communication systems in the wild is to identify the acoustic characteristics of a signal. Categorisations of animal acoustic signals, by humans, have traditionally been based on spectrographic representations of the acoustic signal (Thorpe, 1954). Studies investigating bat social call structure have described individual elements of the call through visual examination of the call sonogram (Fenton, 1976; Barclay et al., 1979; Barclay and Thomas, 1979; Pfalzer and Kusch, 2003; Furmankiewicz, 2004; Jahelkova et al., 2008). This study also described individual elements of the calls through visual examination of the call structure, and found three distinct groups of social calls which could be objectively defined on the basis of their sonogram properties. However, 96.66% of calls had a similar basic call structure, an FM sweep descending in frequency from (mean values 52kHz to 14kHz). This similar structure is in contrast to other species' social calls, in particular CF-FM bats, the moustached bat *Pteronotus parnellii* (Kanwal et al., 1994), and horsehoe bats *Rhinolophus* sp.(Matsumura, 1979; Andrews et al., 2006; Ma et al., 2006), whose communication calls have a more variable call structure, including calls that are unique to the repertoire of CF-FM bats and not found in other bat species with more general echolocation strategies (Ma et al., 2006). However, although Type A calls were broadly similar in structure, there was a lot of variation in the acoustic parameters measured.

This study describes social call structure in one context (i.e. social calls recorded maternity roost sites). A study examining *P. auritus* social call structure in a variety of contexts, three swarming sites (one city park and two abandoned mines) and a maternity colony situated in a church attic, recorded 870 calls that were classified on the basis of visual appearance into six different call types (Furmankiewicz, 2004). These included V – shaped signals, when two or

more bats chased each other, (similar to the 'Type C' calls described in this study), and undulating FM calls followed by a descending FM sweep (similar to the 'Type B' calls described in this study)'. Furmankiewicz (2004) also noted that *P. auritus* produced 'rhythmic and rapid sequences of two, three or more (up to tens of) single FM type calls one after the other'. The study concluded that there was more diverse vocalisations produced at swarming sites, and that these diverse vocalisations 'probably play a role during the mating seasons of this species during spring and autumn swarming' (Furmankiewicz 2004).

An alternative approach to studying acoustic characteristics of a signal is to extract features directly from the acoustic signal. These features typically represent well-defined properties of audio signals, such as the duration of the call and the frequency bandwidths of the call. The main advantage in using this method is that experimenter bias can be avoided, but it is dependent on the features used, which may not always be extracted correctly by the experimenter. Taking the Type A social calls, and subjecting them to a PCA and cluster analysis, revealed that these calls could be further divided into subgroups or clusters on the basis of their acoustic properties. The best number of clusters ranged from four to six, with six being the strongest overall candidate. Cluster one overlapped with both clusters five and six, indicating that these clusters shared similar acoustic properties. Social calls in clusters five and six are characterised by a long duration (12.3 and 11.1 ms), low end frequencies (12.0 and 13.2 kHz), similar frequencies of maximum energy (19.6 kHz) but differ predominately in their start frequencies (46.8 and 52.3 kHz). It may be that these calls should actually be considered part of the same cluster as high frequency components of the calls may have been missing, as they attenuate more rapidly as they travel through the air (Arch and Narins, 2008). Furthermore, as a result of the directionality of many bat calls, the angle between the bat's mouth and the microphone of the recording equipment will affect signal

characteristics (Waters, 2001). One further potential issue with the low frequency sounds recorded was that the frequency response curve of the microphone has a low frequncy roll-off and fails to record frequencies below about 10kHz (indicated in Appendix A.4.2.2). The Knowles microphones are a commonly used microphone in commercial bat detectors as they produce a reliable frequency response curved in frequencies over 20kHz (Lars Petterson pers.comm. The lack of sensitivity to frequencies below 10kHz can be seen in Figure A.4.2.2 in the abrupt cut off in the properties of calls below this frequency. A total of 202 social calls (approximately 1.76% of the call database) had minimum frequencies below 10kHz. This means that the data presented for the minimum frequency (Fmin) are unreliable and that statistics that rely on this value are uncertain.

There was, in general, good agreement between the acoustic parameters of the clusters with both the model-based cluster analysis and the k-means cluster analysis. This agreement between the two clustering methods suggests that different clusters represent different call types even though the misclassification errors as a result of overlap are high. Therefore, a total of at least eight different *P. auritus* social call types were identified at maternity roost sites (six from 'Type A' calls and one each from 'Type B' and 'Type C'). The calls from Type B and Type C were not subjected to cluster analysis due to the low sample size and infrequency of occurrence. These calls were only recorded later in the season at maternity roosts (see Chapter 3).

Cluster analysis has been used to classify dolphin *Tursiops truncastus* whistles (McCowan, 1995) and humpback whale *Megaptera novaeangliae* social sounds (Stimpert et al., 2011). Wood et al., (2005) also used a model-based cluster analysis approach to classify elephant *Loxodonta africana* rumbles, where information pertaining to which elephant produced the

rumble was not available. They found that measuring the physical properties of 663 elephant rumbles, and subjecting these to cluster analysis, revealed three different types of rumbles that differed by their acoustic parameters, and these rumbles types were significantly associated with different behaviours. In this study it was not possible to associate call type with specific behaviour, but all the calls were produced in the vicinity of the roost site, and many were produced in the presence of other colonies members, in particular during swarming behaviour at dawn (see Chapter 3).

A number of studies have described vocal syntax for birds (Podos et al., 1999; Clucas et al., 2004; Berwick et al., 2011) whereas, by comparison, singing behaviour and syntax are exceedingly rare in mammals (Bohn et al., 2009). Evidence of patterns in call sequences has been described for moustached bats *Pteronotus parnelli* (Kanwal et al., 1994), horseshoe bats *Rhinolophus ferrumequinum* (Ma et al., 2006), and Mexican free-tailed bats *Tadarida brasiliensis* (Bohn et al., 2008). However, these studies did not go beyond determining that the order of call elements was non-random. Markov chains have been used to analyse and describe vocal syntax in birds (Ficken et al., 1994) and cetaceans (McCowan, 1995). More recently, a study by Bohn et al. (2009) found evidence using Markov chain processes that male Brazilian free-tailed bat song *Tadarida brasiliensis* produce songs with the same four types of syllables and the same three types of phrases across two regions. In this study no evidence was found that *P. auritus* produce sequences or phrases of calls at maternity roosts sites, only that calls are produced in sequences of similar call types (i.e. a call from one cluster is more likely to be followed by a call from the same cluster rather than a call from a different cluster). However, the Markov chain analysis conducted in this study was limited to the analysis of couplets and it may be that higher order sequences do exist.

There is evidence that communication calls in animals provide sufficient information for recognition of individual identity (Reby et al., 1998; Clark et al., 2006; Sproul et al., 2006). Studies investigating the variation in echolocation calls have found evidence that echolocation calls may provide information about the caller's identity (Kazial and Masters, 2004; Kazial et al., 2008a; Kazial et al., 2008b; Yovel et al., 2009; Voigt-Heucke et al., 2010), but, this may be species specific as not all studies have demonstrated such individual specific signatures for echolocation calls (Siemers and Kerth, 2006). Social calls, by contrast, are deemed to have primarily a communicative function, are more structurally complex, and therefore offer the possibility to encode both individual and contextual information (Russ and Racey, 2007; Carter et al., 2008; Melendez and Feng, 2010). Although this study did not examine aspects of individual recognition, as it was not known who or how many bats were calling, the variation recorded in the Type A social calls may be more specifically related to individual calling parameters as opposed to specific call types. The calls were however, recorded from 20 different roost sites in three separate geographic areas, which allows for an assessment of whether all call types occurred at all of the roosts or whether they were specific to roost types. This will be examined further in Chapter 3.

2.5 – Summary

Chapter 2 aimed to describe the structure of *Plecotus auritus* social calls recorded at maternity roost sites in Sussex. A total of 11,484 social calls were recorded and referred to as Type A, B and C.

- Type A calls (approximately 96% of the calls recorded) shared the same basic pattern (a FM sweep descending in frequency from a mean of approximately 52kHz to about 14kHz) but within this call type there was a lot of variation and principal component analysis and model based cluster analysis identified six separate clusters.

- Type B calls differentiated objectively from Type A calls on the basis of their starting frequency being lower than the maximum frequency within the call.
- Type C calls could be differentiated from Type A and Type B calls on the basis of their end frequency being higher than the minimum frequency within the call.
- Markov chain analysis of Type A calls found that for each of the six Type A calls classified was more likely to be followed by a repeat of the same call type than by any other call type.

Figure 2.1: Ultrabat recording equipment. The numbered parts are as follows:

1. PC (Torch Mini 12v pc (ESaw Ltd., London)
2. Timer / Control Unit
3. Power Supply – 12V Battery shown
4. Microphone amplifier (Knowles EK23132)
5. Waterproof case

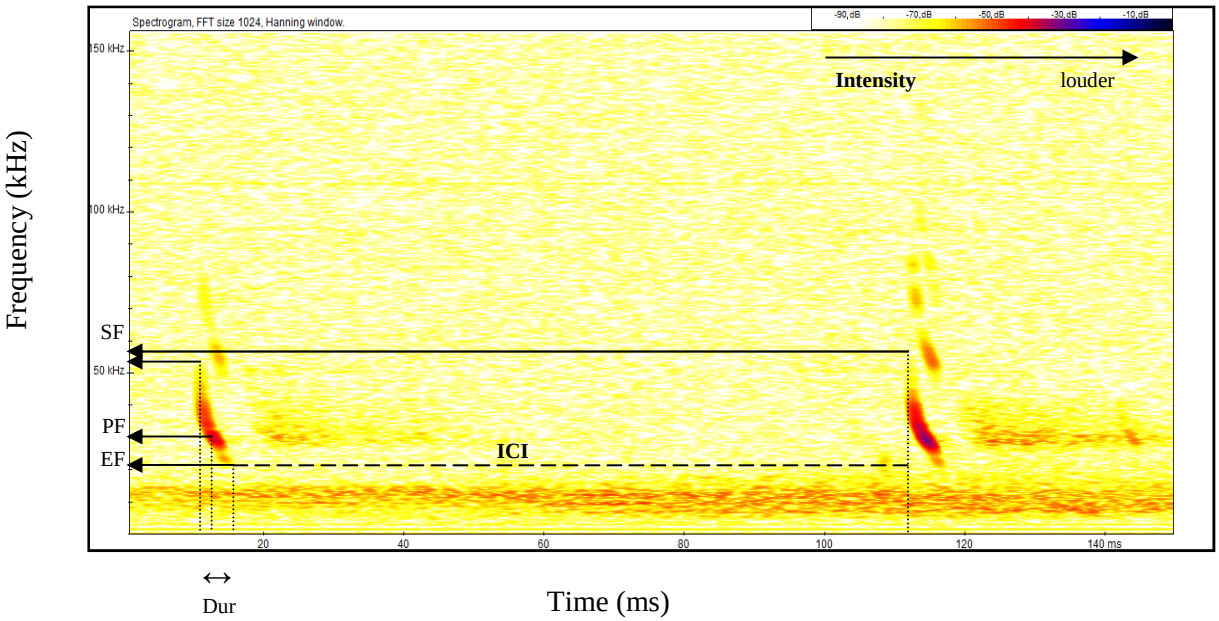

Figure 2.2: Sonograms of two typical Brown long-eared bat social calls. **SF** = the start frequency of the call, also here the maximum frequency (**FMax**), **PF** = peak frequency of maximum energy, **EF** = end frequency of the call, also here the minimum frequency (**FMin**), **Dur** = the duration of the call, **ICI** = inter-call interval between the end of one social call and the beginning of the next, Intensity = loudness of the call.

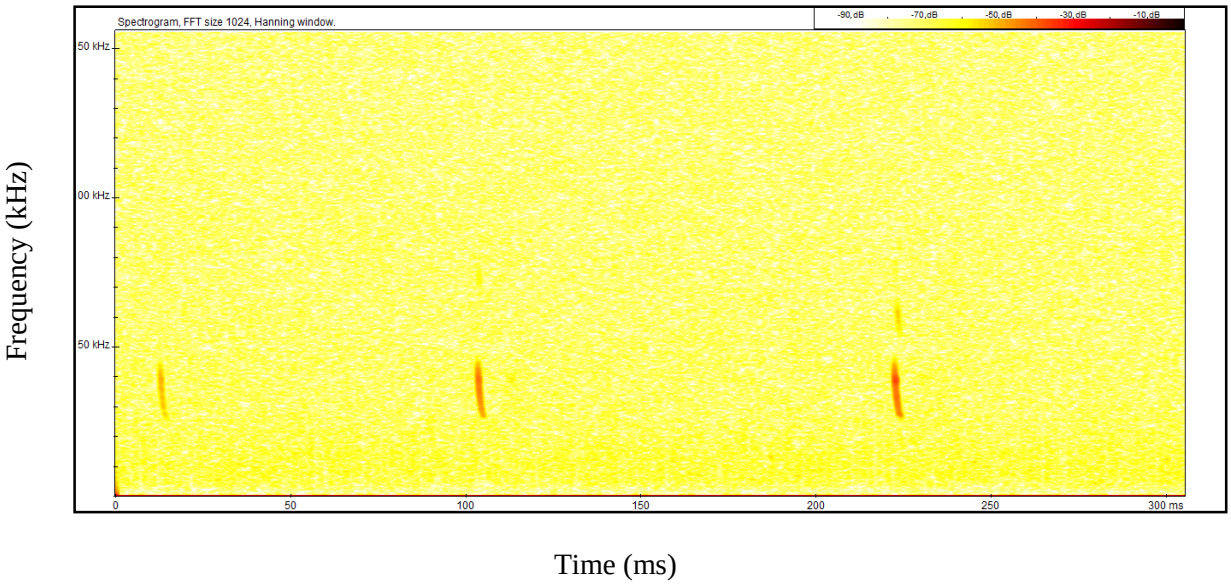

Figure 2.3: Sonograms of three pulses of typical Brown long-eared bat echolocation calls.

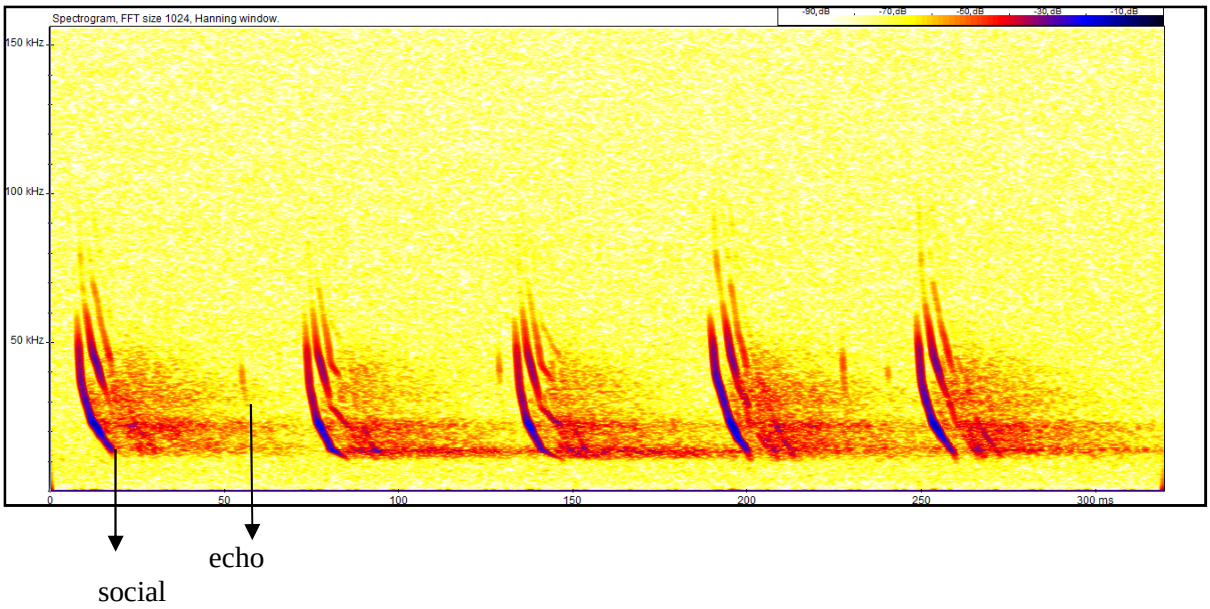

Figure 2.4: Sequence of *P. auritus* social calls, comprising of five social calls in the sequence. There is also clearly evidence of a second bat present as there is echolocation of *P. auritus* interspersed with the social calls.

	Mean	Minimum	Maximum	Std. Dev	Std. Error	Variance
Duration (ms)	10.61	1.43	19.80	2.11	0.02	4.43
Fmin (kHz)	13.95	8.00	41.20	3.81	0.04	14.58
Fmax (kHz)	51.97	19.60	73.20	4.87	0.05	23.78
Fstart (kHz)	51.97	19.60	73.20	3.81	0.05	23.78
Fend	13.95	8.00	41.20	3.81	0.04	14.58
Fmaxe	20.68	10.70	51.80	5.65	0.06	31.93
ICI (ms)	235972	0	19020000	1171940	11146	1.373E+12

Table 2.1: Descriptive statistics for the acoustic parameters of Type A social call (n = 11,101) recorded at maternity roosts.

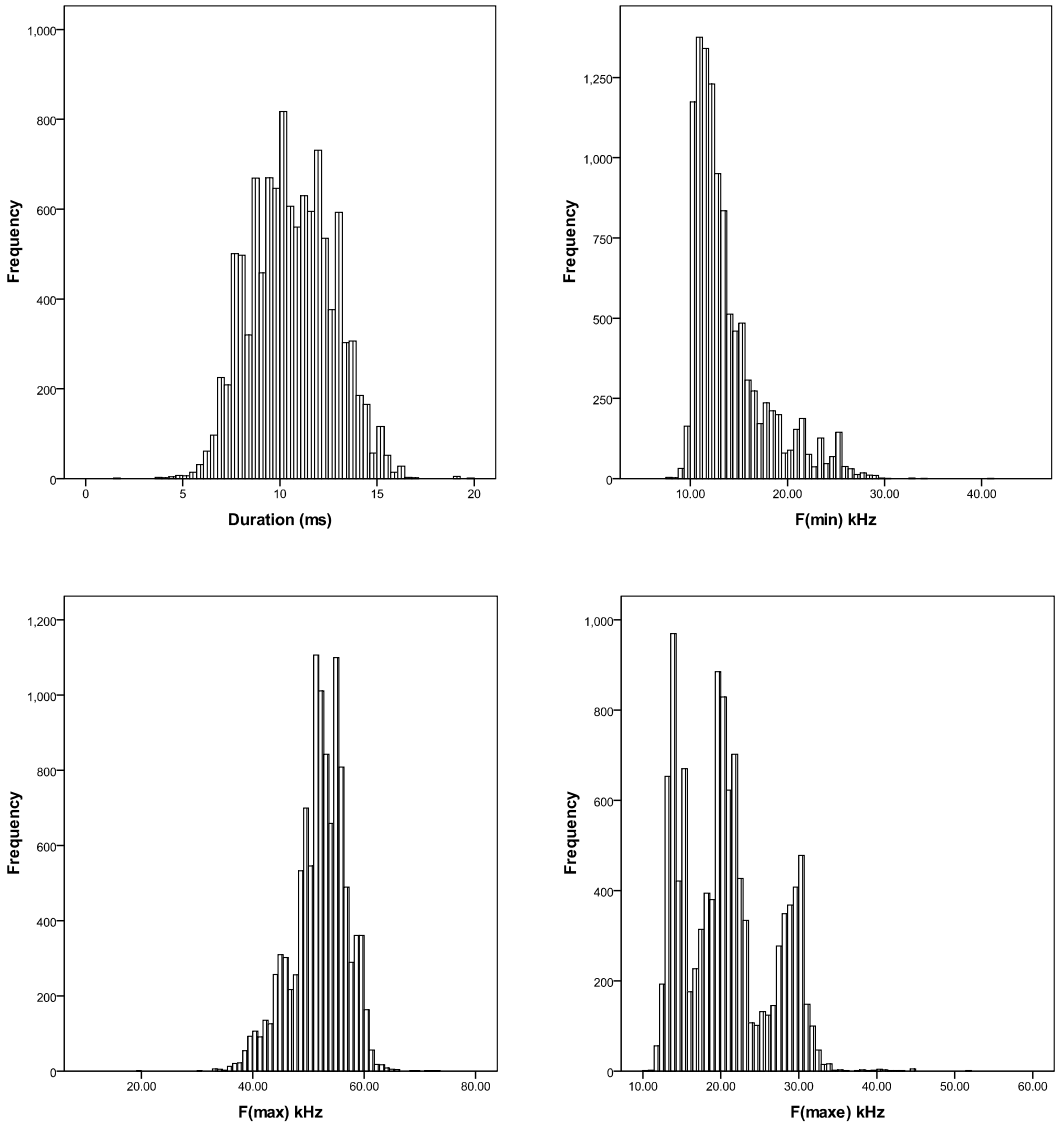

Figure 2.5: Variation in four acoustic parameters for Type A social call (n = 11,101) recorded at maternity roosts.

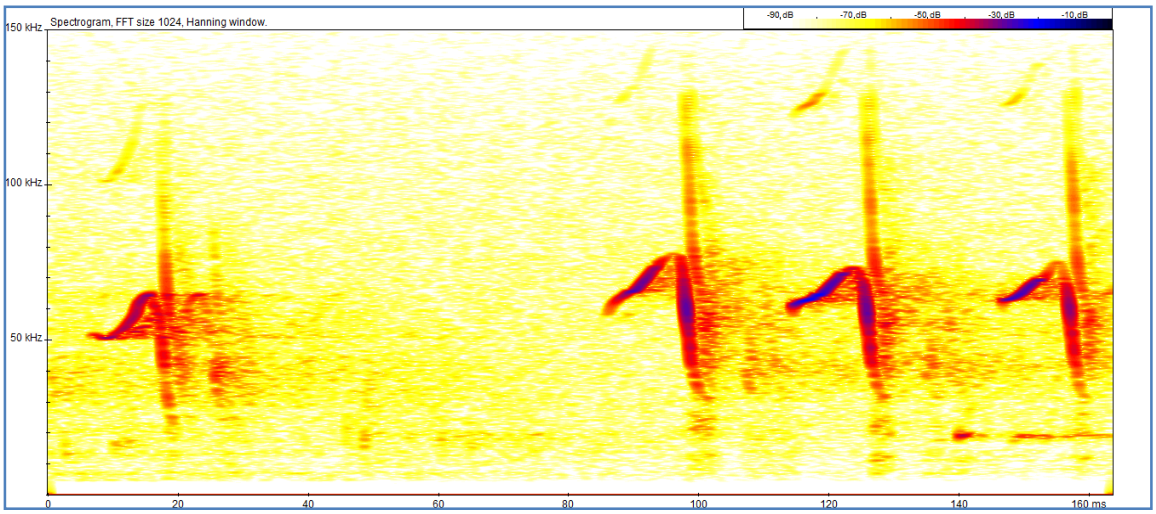

Figure 2.6: Series of four 'Type B' social calls, characterised by undulating upward FM sweep, followed by a descending FM sweep.

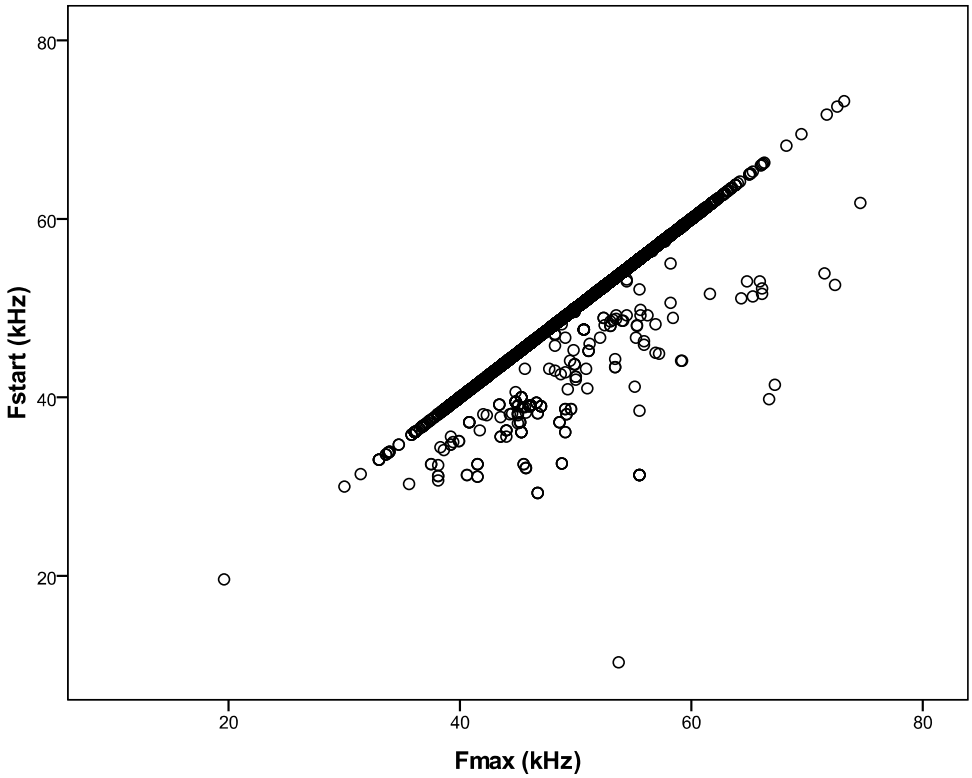

Figure 2.7: Correlations between the start frequency and maximum frequency of *P. auritus* social calls at maternity roost sites. The majority of calls had equal start and maximum frequency values, but Type B calls were characterised by a lower starting frequency value compared to the maximum frequency value recorded within the call structure.

	Mean	Minimum	Maximum	Std. Dev	Std. Error	Variance
Duration (ms)	10.01	5.20	22.90	2.60	0.18	6.76
Fmin (kHz)	17.45	10.10	37.20	5.08	0.35	25.85
Fmax (kHz)	49.15	35.60	74.60	6.84	0.47	46.84
Fstart (kHz)	41.24	10.30	61.80	7.20	0.49	51.87
Fend	17.45	10.30	37.20	5.08	0.35	25.85
Fmaxe	26.70	12.00	63.50	9.08	0.62	82.41
ICI (ms)	62546	0	9420000	9.17114E+5	62546	8.411E+11

Table 2.2: Descriptive statistics for the acoustic parameters of Type B social call (n = 215) recorded at maternity roosts.

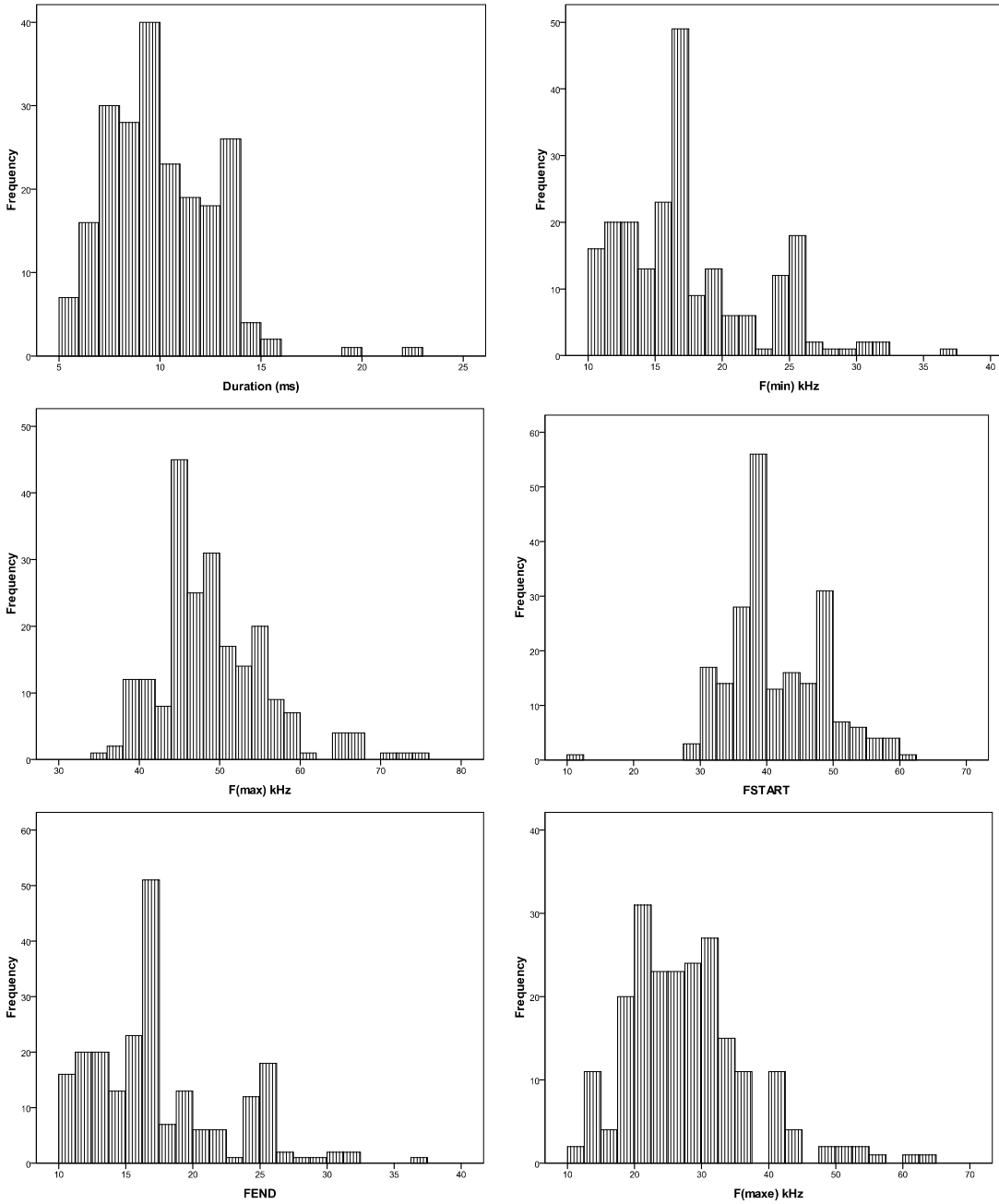

Figure 2.8: Variation in six acoustic parameters for Type B social calls recorded at maternity roosts (n = 215).

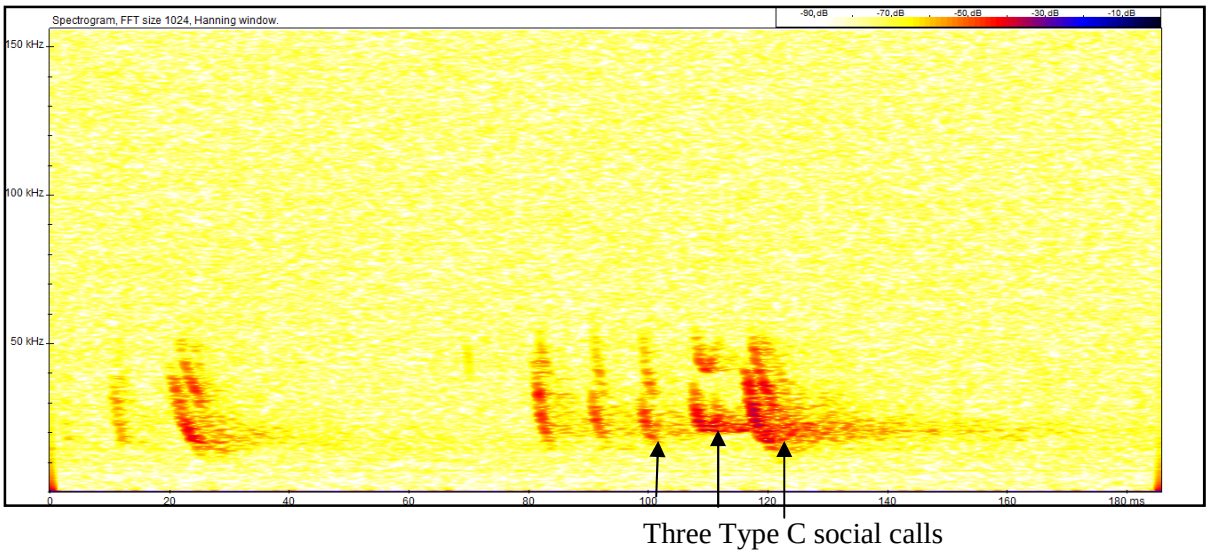

Figure 2.9: Series of seven social calls, three of which are classified as Type C calls, characterised by an upward FM sweep at the end of the call.

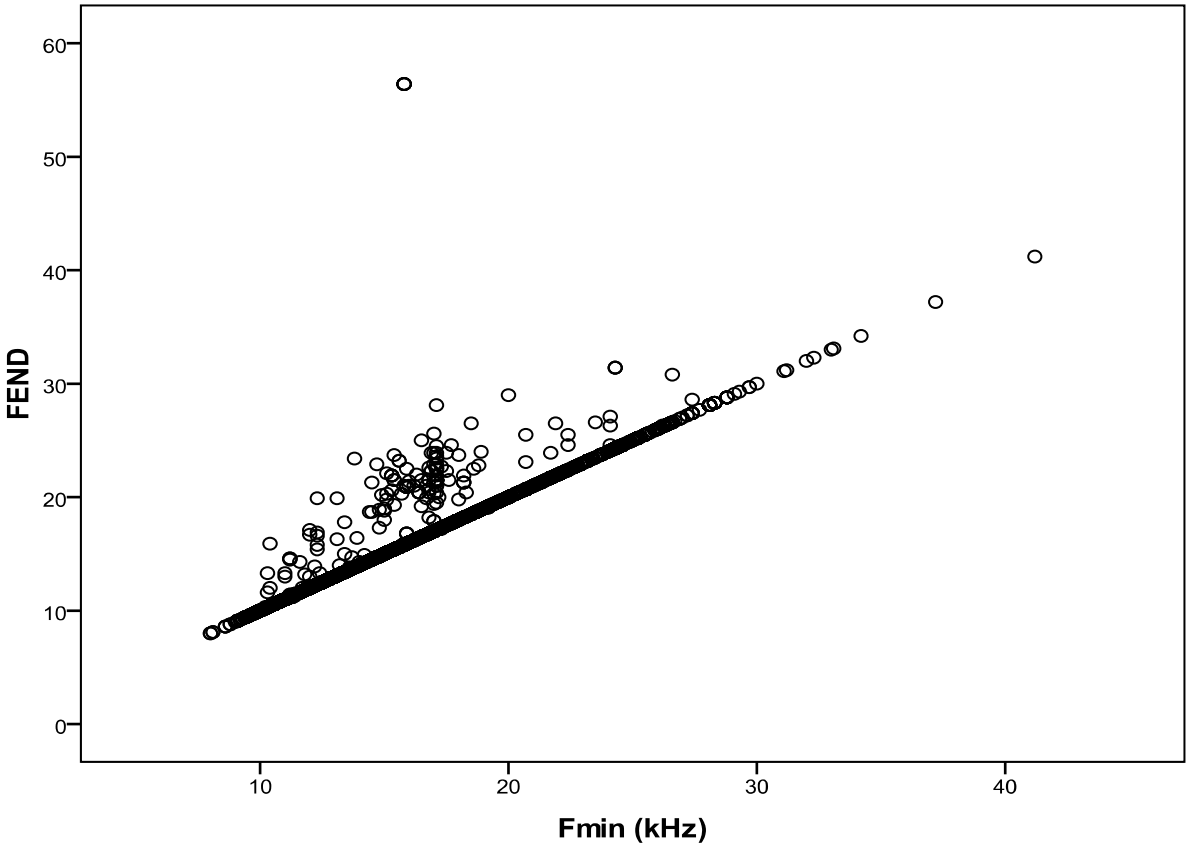

Figure 2.10: Correlation between the end frequency and minimum frequency of *P. auritus* social calls at maternity roosts. The majority of calls had equal end and minimum frequency values, but Type C calls were characterised by an end frequency that was higher than the minimum frequency.

	Mean	Minimum	Maximum	Std. Dev	Std. Error	Variance
Duration (ms)	9.29	4.10	13.3	1.75	0.14	3.06
Fmin (kHz)	16.27	10.30	27.40	3.13	0.24	3.13
Fmax (kHz)	49.87	31.40	59.40	6.11	0.47	37.28
Fstart (kHz)	49.87	31.40	59.40	6.11	0.47	37.28
Fend	20.55	11.40	31.40	4.04	0.31	16.29
Fmaxe	23.23	14.90	44.30	5.19	0.40	26.98
ICI (ms)	134833	0	7740000	8.11152E+5	62768	6.580E+11

Table 2.3: Descriptive statistics for acoustic parameters of Type C social calls (n = 168).

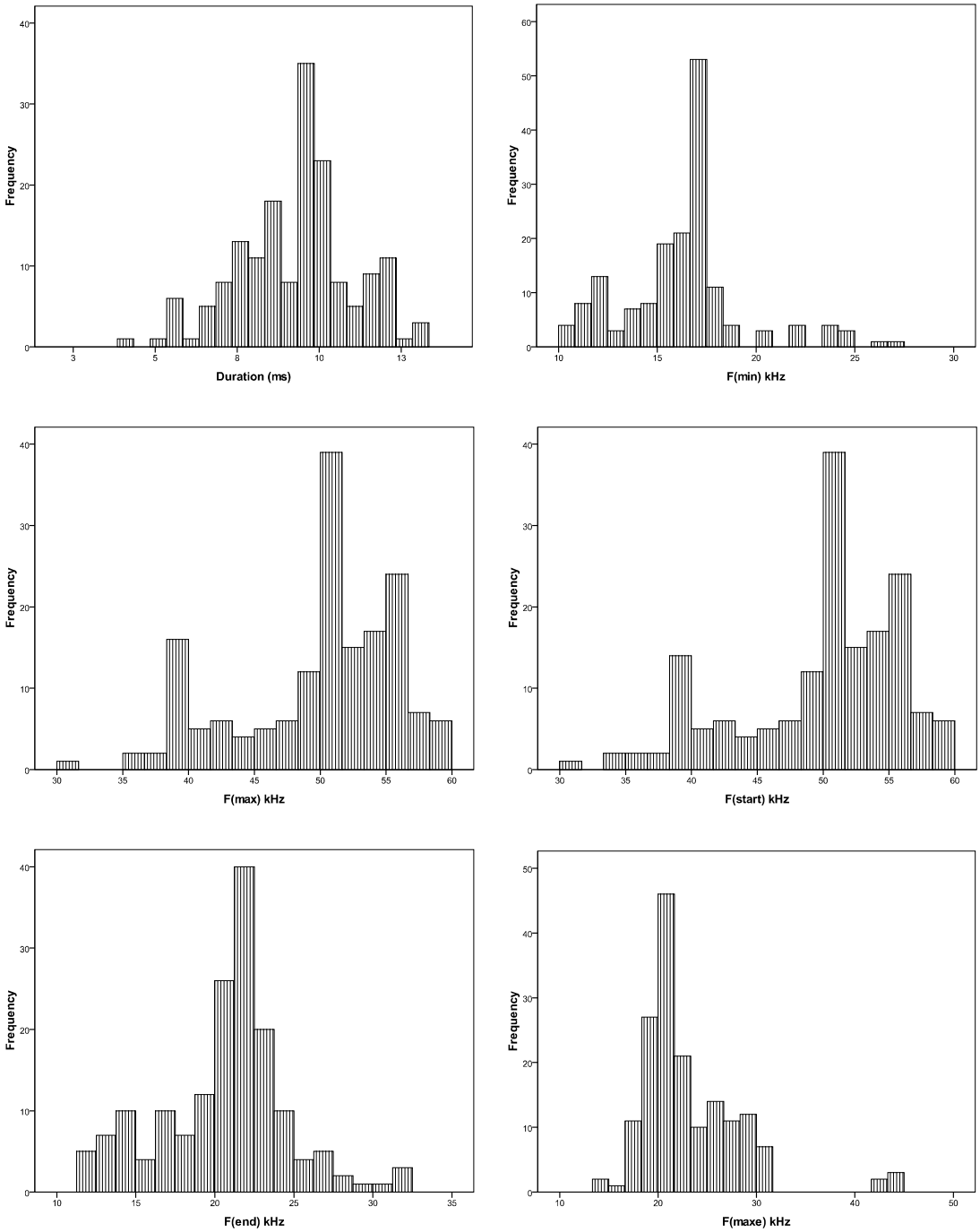

Figure 2.11: Variation in acoustic parameters for Type C social calls recorded at maternity roosts (n = 168).

	Duration (ms)	Fmin (kHz)	Fmax (kHz)	Fstart (kHz)	Fend (kHz)	Fmaxe (kHz)
Duration (ms)		r = -0.526*	r = 0.097	r = 0.099	r = -0.526*	r = 0.596*
Fmin (kHz)			r = -0.088	r = -0.099	r = 0.977*	r = 0.736*
Fmax (kHz)				r = 0.989*	r = 0.088	r = -0.043
Fstart (kHz)					r = -0.099	r = -0.055
Fend (kHz)						r = 0.737*
Fmaxe (kHz)						
	*Correlation is significant at 0.01 level (2-tailed)					

Table 2.4: Spearmans rank coefficient of correlation between six acoustic parameters of all social calls recorded at maternity roosts.

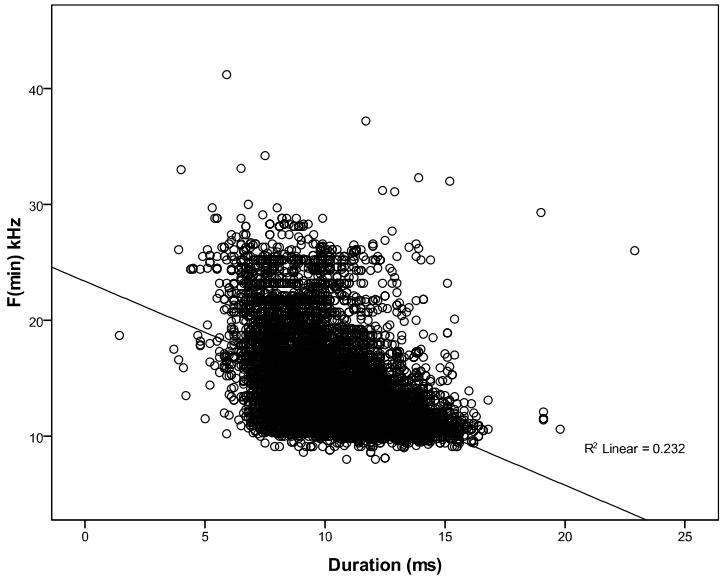

Figure 2.12(a): Correlation between duration and minimum frequency for all social calls at maternity roosts.

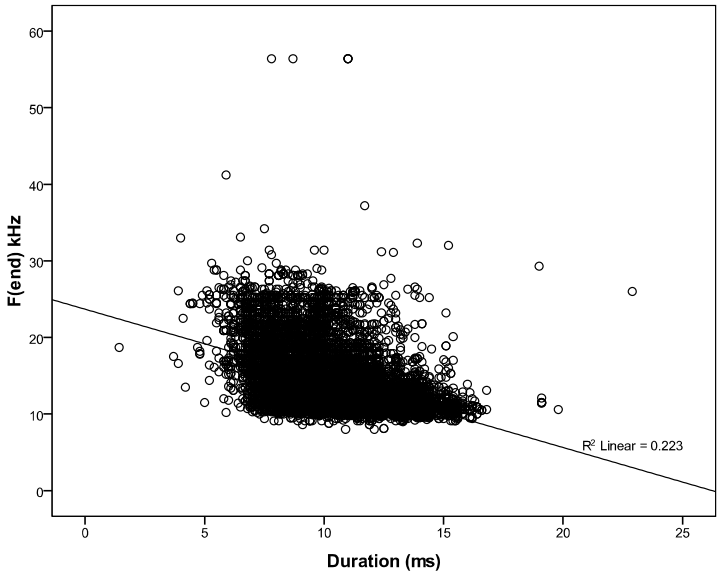

Figure 2.12(b): Correlation between duration and end frequency for all social calls at maternity roosts.

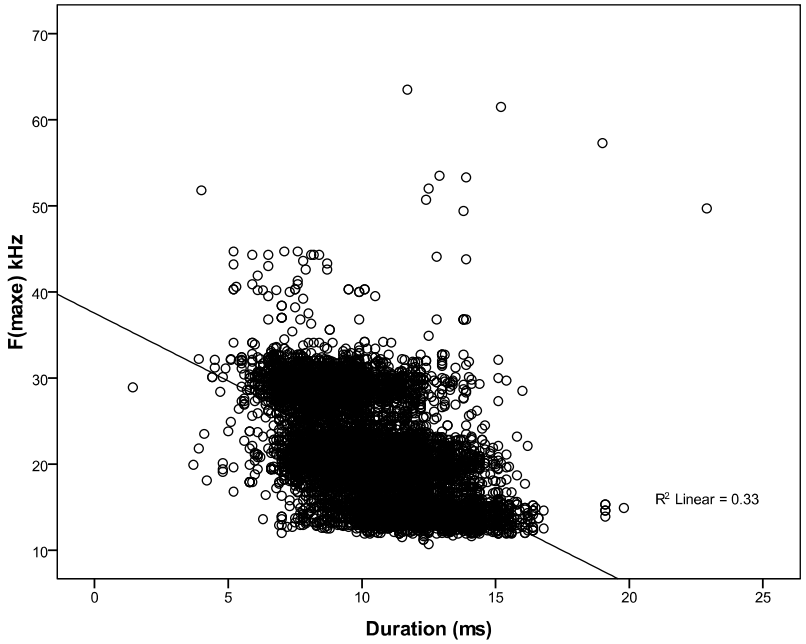

Figure 2.12(c): Correlation between duration and frequency of maximum energy for all social calls recorded at maternity roosts.

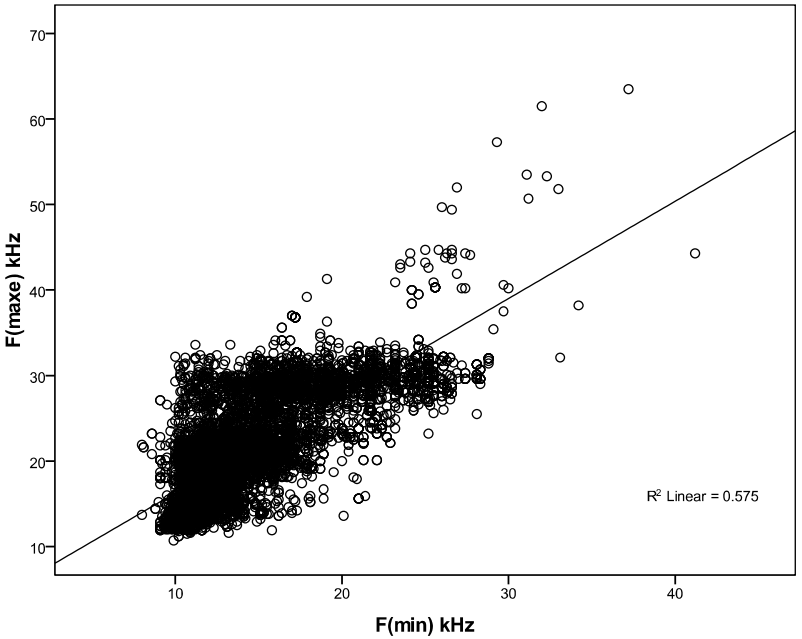

Figure 2.12(d): Correlation between minimum frequency and frequency of maximum energy for all social calls recorded at maternity roosts.

Component	Initial Eigenvalues			Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings[a]
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %	Total
1	3.089	51.490	51.490	3.089	51.490	51.490	3.051
2	1.909	31.819	83.309	1.909	31.819	83.309	2.003
3	.655	10.922	94.231				
4	.283	4.713	98.944				
5	.032	.538	99.483				
6	.031	.517	100.000				

Table 2.5: Results of the Principal Component analysis of 11,464 *P. auritus* social calls. The first two components account for approximately 83% of the variance in the data.

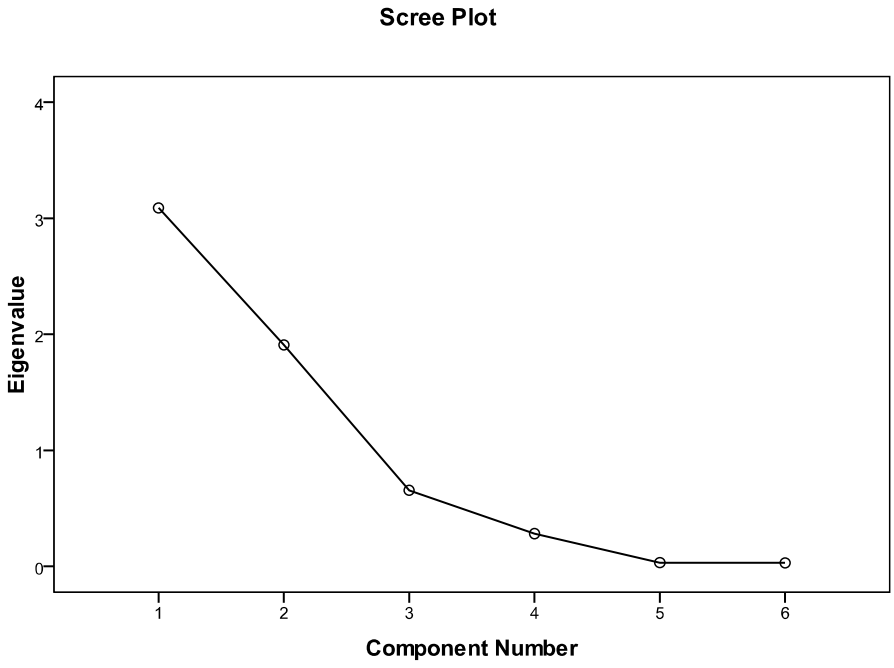

Figure 2.13: Scree plot of the eigenvalues from the Principal Component Analysis in descending order of magnitude. There is no clear single point of inflexion.

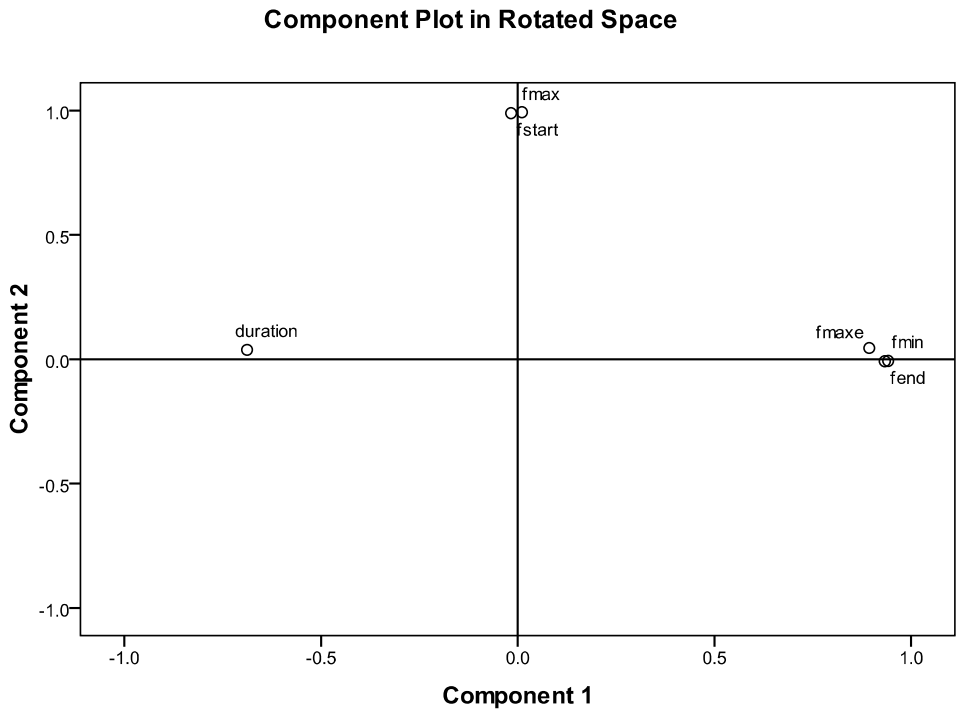

Figure 2.14: Location of the six acoustic parameters in relation to the two principle components identified by the analysis..

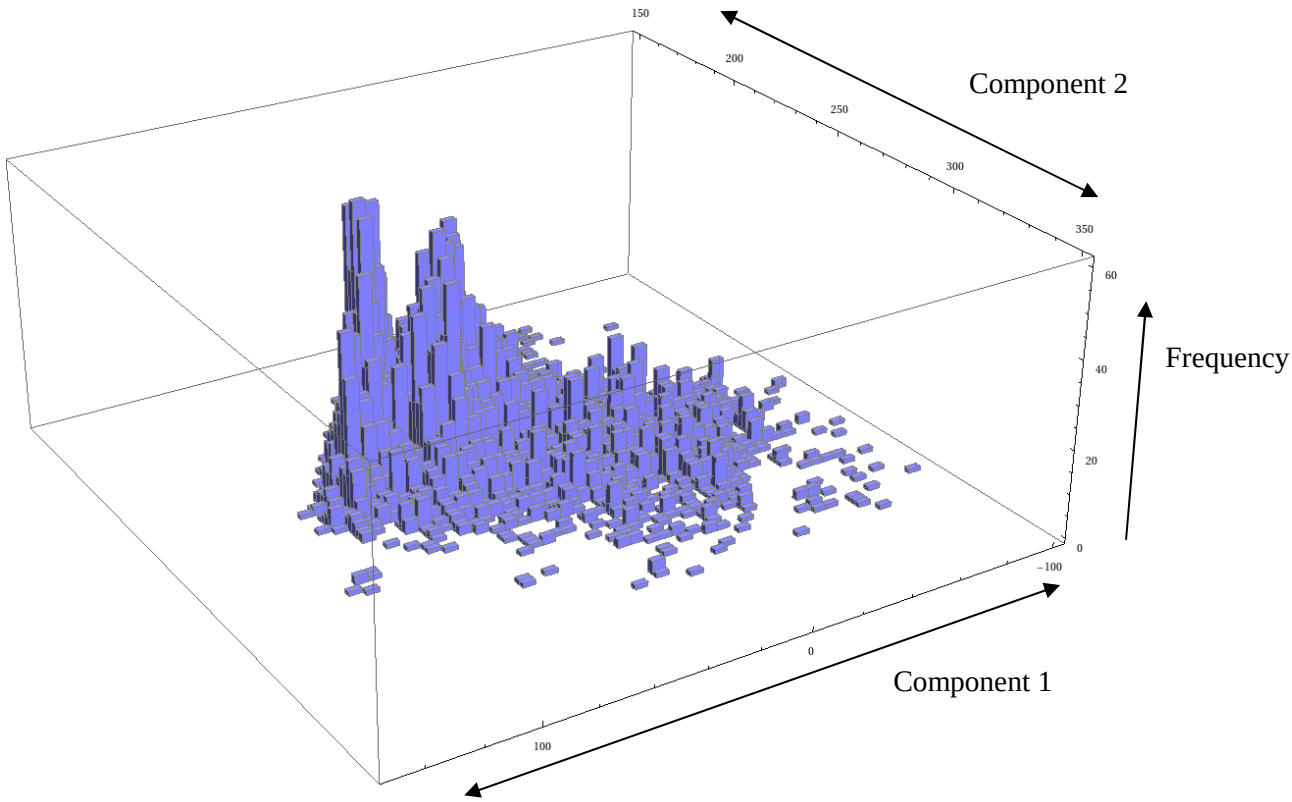

Figure 2.15: Frequency distribution of the first two components. The histogram demonstrates the overlap in parameters between calls.

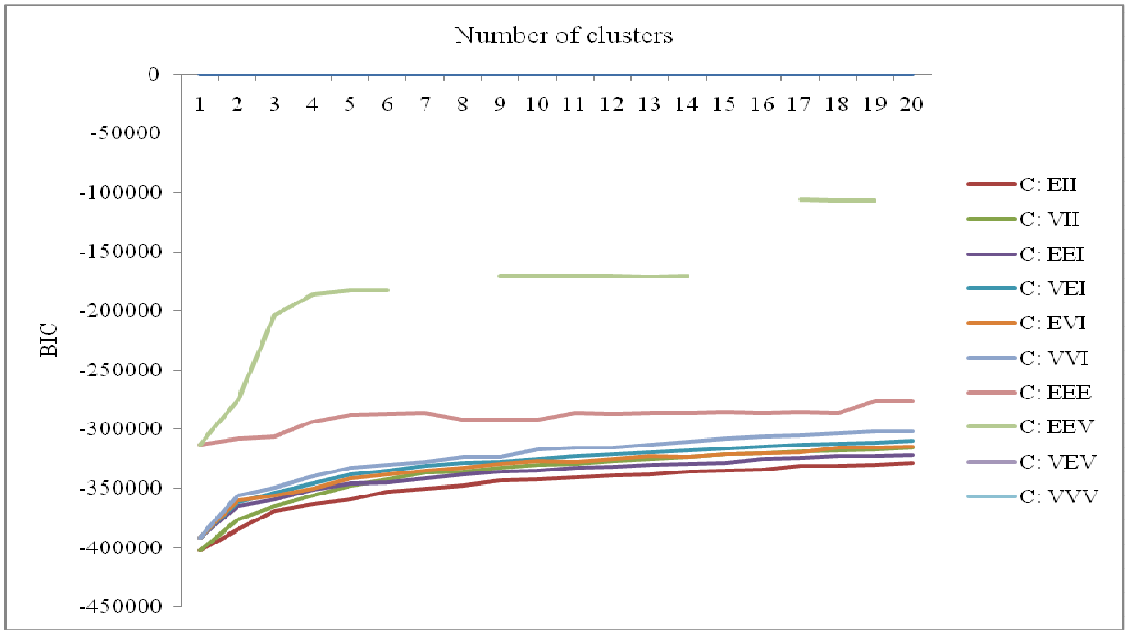

Figure 2.16: Bayesian information criterion values for 1 to 20 clusters for *P. auritus* call data. The 10 models tested (described in Farley and Raftery 2002) found that the EEV model was the best fit. However, missing values have occurred as a result of one or more estimated covariance matrices being too close to singularity (Fraley and Raftery, 1998) .

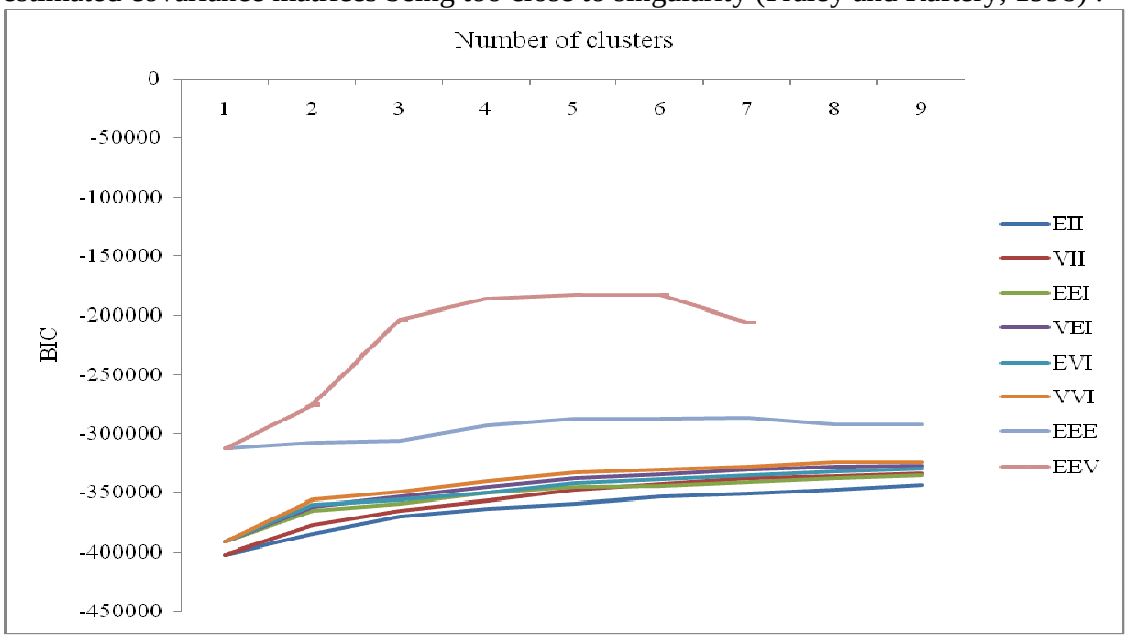

Figure 2.17: Bayesian information criterion values for 1 to 9 clusters for *P. auritus* PCA factor data. The 10 models tested (described in Farley and Raftery 2002) found that eight models fitted the data but the EEV model again was the best fit. The model reaches asymptote at four (or arguably five) clusters and begins to decrease after six clusters. Therefore, six clusters using the EEV model best fitted the dataset.

Run number	Number of clusters	Model	BIC value
1	6	EEV	-170220
	5	EEV	-170336
	4	EEV	-170556
2	9	EEE	-169987
	6	EEV	-170231
	5	EEV	-170344
3	6	EEV	-171211
	9	EEE	-171302
	4	EEV	-171399
4	7	EEV	-171399
	6	EEV	-171404
	4	EEV	-172509
5	5	EEV	-170356
	8	EEV	-170399
	6	EEV	-170443

Table 2.6: Results of the highest three Bayesian information criterion values for five runs of cluster analysis using a random sub-set of 75% of the acoustic parameters.

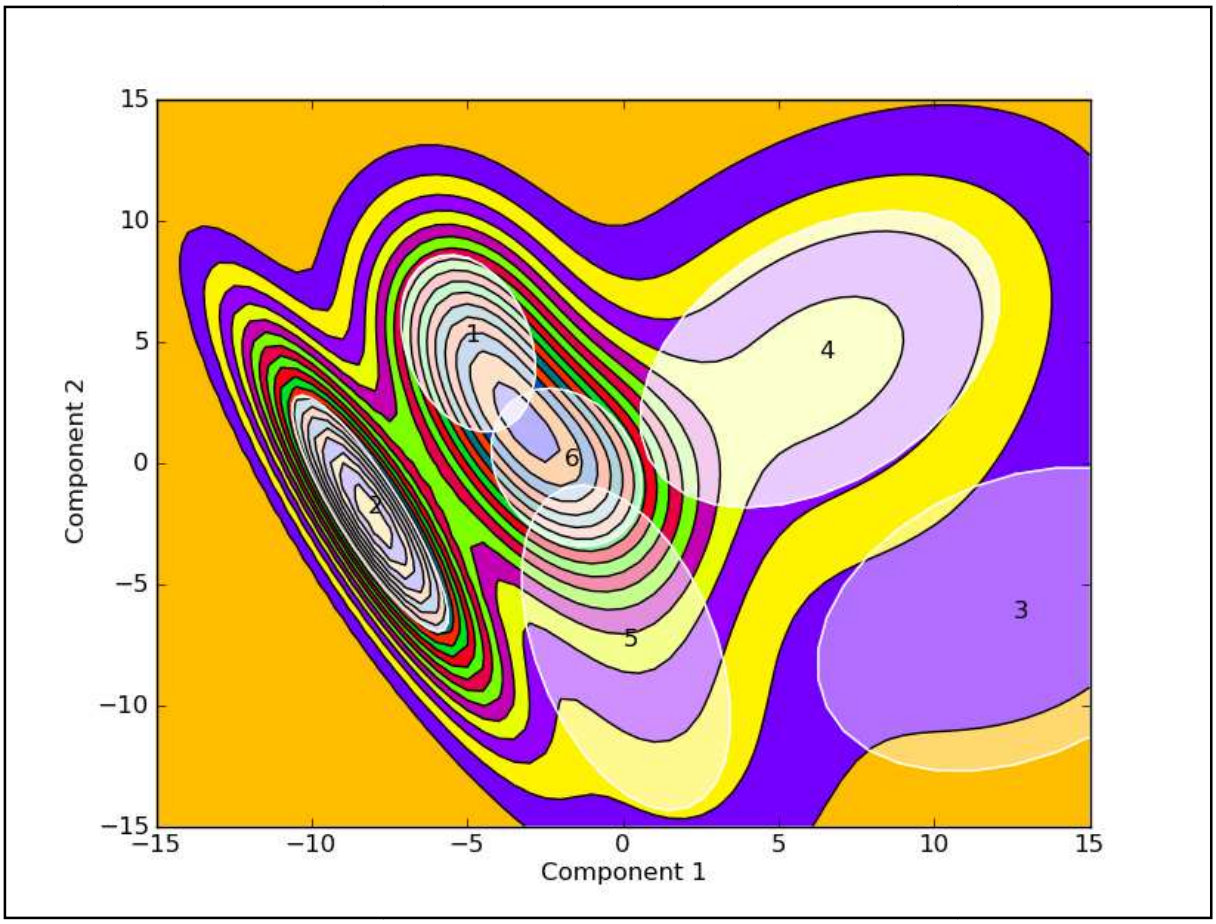

Figure 2:18: A representation of the six Gaussian clusters fitted to the data distribution. The contours represent frequency, whereby closely fitted contours indicate a high frequency of similar data points, such as the dense clusters one, two and six. Clusters three, four and five are more widely dispersed containing calls that are less similar to one another.

Cluster	Archetype call number	Duration	Fstart	Fmax	Fend	Fmin	Fmax(e)	No. of calls
1	832	9.8	56.3	56.3	56.3	10.6	21.8	2084
2	5470	12.4	53.1	53.1	53.1	10.6	14.5	2624
3	9640	9.0	43.7	43.7	43.7	15.0	31.2	842
4	10746	10.9	52.5	52.5	52.5	17.2	27.2	2411
5	4411	12.3	46.8	46.8	46.8	12.0	19.6	1168
6	10629	11.1	52.3	52.3	52.3	13.2	19.6	1972

Table 2.7: The acoustic parameters of the archetype social call with the highest probability of coming from each of the six clusters. The call number is the number of the call in the database. The number of calls is the total number of calls in each of the six clusters.

Clusters	1	2	3	4	5	6
1	0.746	0.018	0.001	0.048	0.035	0.152
2	0.029	0.889	0.001	0.021	0.041	0.188
3	0.000	0.001	0.849	0.113	0.036	0.000
4	0.010	0.000	0.064	0.850	0.028	0.047
5	0.003	0.047	0.108	0.100	0.658	0.089
6	0.144	0.007	0.004	0.149	0.151	0.542

Table 2.8: Probability values for the classification calls from one clusters with another cluster type. For example, there was a 2.9% chance of classifying a Cluster 2 calls as Cluster 1 and a 1.8% chance of classifying Cluster 1 call as Cluster 2.

Cluster	Number of calls	Duration	Fmin	Fend	Fmax	Fstart	Fmax(e)
1	2590	10.03	13.40	13.39	51.34	51.34	21.72
2	2400	12.29	11.20	11.20	52.06	52.06	14.47
3	778	8.88	16.06	16.06	45.43	45.43	28.51
4	1525	8.72	19.54	19.54	54.22	54.22	29.26
5	1242	10.83	12.38	12.38	43.58	43.58	18.55
6	2566	11.17	12.38	12.37	57.25	57.25	19.02

Table 2.9: K-means cluster analysis to partition the 11,101 Type A calls into six clusters in which each call belongs to the cluster with the nearest mean.

Cluster	1	2	3	4	5	6
1		21.18	19.50	15.22	12.65	12.28
2			12.86	19.34	16.15	11.55
3				8.87	22.17	8.27
4					22.93	8.97
5						15.33
6						

Table 2.10: K-means cluster analysis Euclidean distances between final cluster centres.

	No Call	1	2	3	4	5	6
No Call	0.000	0.290	0.210	0.080	0.240	0.080	0.100
1	0.010	0.481	0.127	0.013	0.113	0.063	0.193
2	0.012	0.109	0.633	0.010	0.089	0.058	0.089
3	0.013	0.038	0.031	0.476	0.268	0.119	0.054
4	0.006	0.090	0.080	0.080	0.598	0.038	0.107
5	0.009	0.081	0.146	0.094	0.111	0.408	0.151
6	0.005	0.196	0.131	0.023	0.137	0.098	0.410

Table 2.11: Probability values for the matrix of calls in each cluster type. The first column 'no call' represents silence. The values horizontally represent the preceding call and the values vertically represent the following call. As an example, there is a 12% chance of Call 2 following Call 1, and a 10% chance of Call 1 following Call 2.

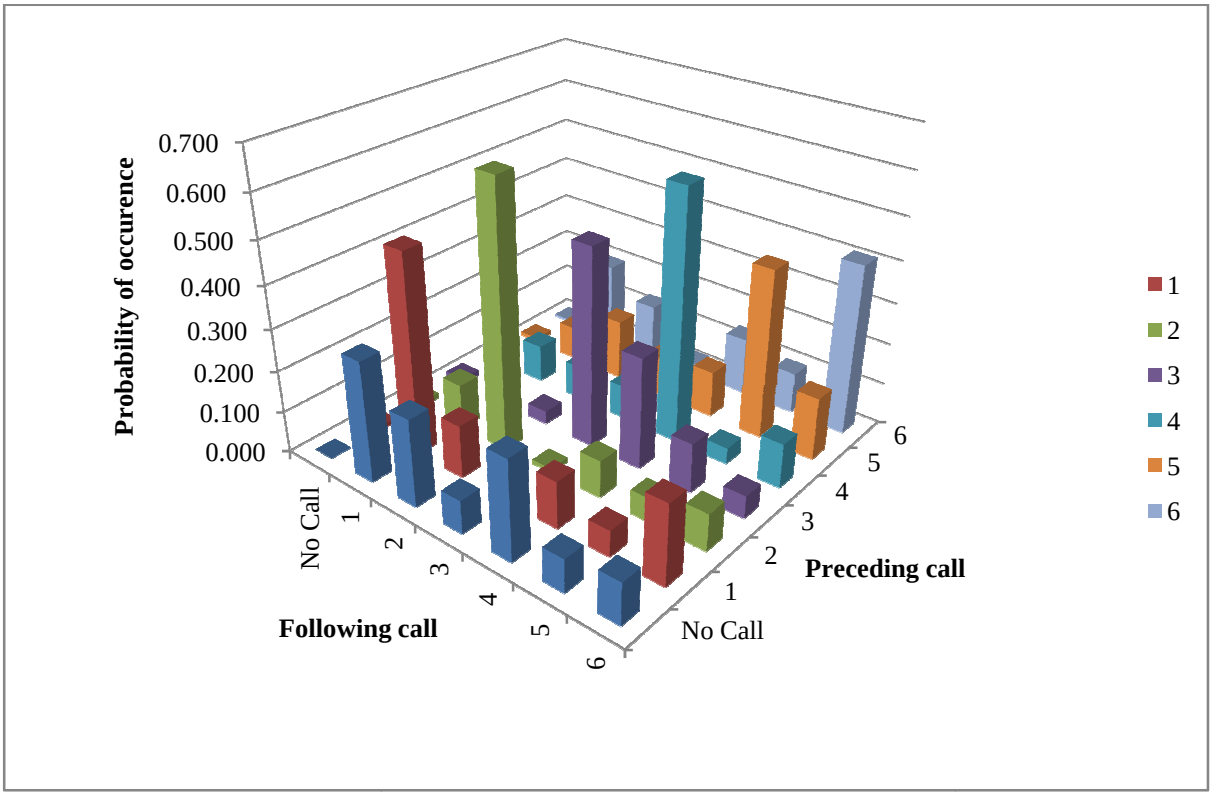

Figure 2.19: Three-dimensional bar chart of a transitional analysis consisting of pairs to show the probabilities with which one call type follows another.

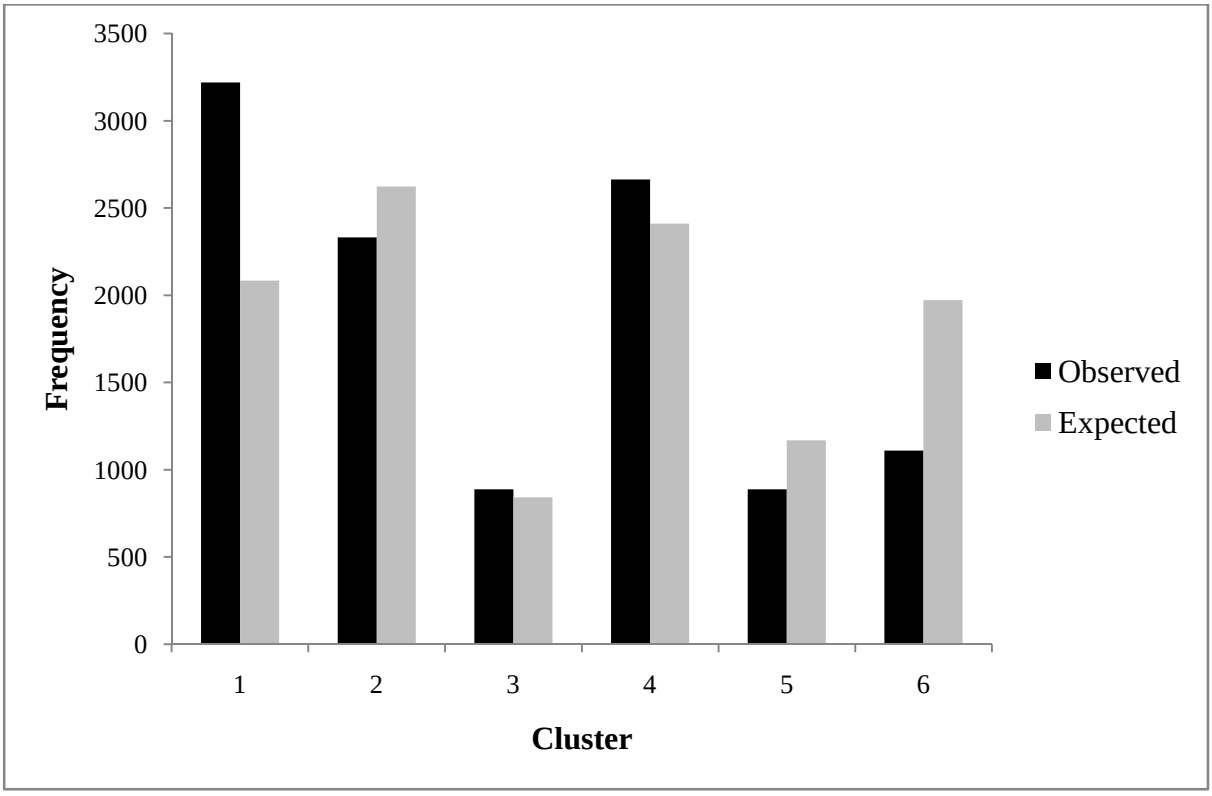

Figure 2.20: Observed and expected frequencies for each call type following silence. Calls type 1 was first more often than expected and Type 6 was first less often than would be expected from their frequency alone.

3.1 - Introduction

Bats occupying the temperate zone can be described as gregarious mammals that aggregate into groups for at least part of their lifecycle and occupy a variety of roost types in both man-made and natural structures. Benefits of group formation include, for example, reduced risk of predation (Parrish and Edelstein-Keshet, 1999) and, also, the reduction of individual energy expenditure in maintaining body temperature (Hayes et al., 1992; Berteaux et al., 1996; Gilbert et al., 2010). Roosts not only offer shelter and protection, but are also used for a variety of purposes including giving birth and raising offspring (Stebbings, 1966; Racey and Swift, 1985; Entwistle et al., 1997; Syme et al., 2001; Garroway and Broders, 2008), mating (Gerell and Lundberg, 1985; Kurta et al., 1993; Sachteleben and von Helversen, 2006) and hibernation (Kunz and Lumsden, 2003). The amount of time spent in roosts means that many social behaviours and interactions are likely to occur within the roost environment and roosts are also important to facilitate information transfer and social interactions within species (Wilkinson 1995; Kerth and Reckardt 2003).

Maternity roosts are usually occupied throughout the active season by females for the purpose of reproduction. Summer (maternity) roosts used by *P. auritus* are most commonly found in buildings, usually in the attic spaces of houses, barns or churches (Entwistle et al., 1997; Swift, 1998) but also in tree cavities (Horáček, 1975; Murphy et al., in prep) and in bat boxes (Boyd and Stebbings, 1989; Fluckiger and Beck, 1995). During the summer *P. auritus* forms stable colonies, comprised of both adult females and males and young of the year (Burland et al., 1999; Entwistle et al., 2000). *P. auritus* is rare amongst temperate zone bats in that adult males are regularly found with females in maternity colonies. Counts of bats

emerging from a maternity colony roosts are a common method of monitoring *P. auritus* populations (Bat Conservation Trust 2007).

Dawn swarming at the entrance to summer maternity roosts has been described for a number of species of bat (McAney and Fairley, 1988; Shiel and Fairley, 2000; Kanuch, 2007). Swarming behaviour occurs when bats return to the roost after foraging, either early on after their first flight or just before dawn when they enter the day roost. Bats fly in wide circles at the entrance to the roost, sometimes pursuing one another (Shiel and Fairley, 2000). Often they appear to land and take off again multiple times before entering the roost. Several hypotheses have been put forward for the function of this behaviour but none have been tested. Proposed functions include information transfer about good foraging areas that night (Wilkinson, 1995; Wilkinson and Boughman, 1998), advertisement of roosting locations (Chaverri et al., 2010; Schöner et al., 2010), novel roost sites (Kerth and Reckardt, 2003), or re-affirmation of social bonds between roost mates and anti-predatory behaviour (Wilkinson, 1995).

The role of social calls in actively maintaining associations among roost members has received little attention. If one of the functions of social calls produced at roost sites is to facilitate group cohesion then it would be expected that the number of calls recorded at roost sites would be positively correlated with colony size, as all bats would vocalise as part of the bonding process. A study on the relationship between social vocalisations and group size in Hawaiian humpback whale *Megaptera novaeangliae* found that the rate of vocalisation was positively correlated with group size (Silber, 1986). Conversely, if only one or a few ‘dominant’ individual called at roost sites, providing information about roosting sites or good foraging areas, then it would be expected that there would be no relationship between colony

size and the number of social calls recorded. For example, dominant green woodhoopoes *Phoeniculus purpureus* vocalise when they leave a group for a new foraging site, thus advertising their departure and attracting the attention of other group members to follow (Radford, 2004). Additionally, if social calls at roost sites function to reunite adult females with off-spring when adult females return to the roost after foraging, then seasonal and temporal patterns should be apparent around the birthing and rearing period. Therefore, I wanted to examine whether there are temporal and seasonal patterns of calls and sequences produced at *P. auritus* maternity roost sites.

3.2 - Methods

3.2.1 – Roost locations

Ten summer roost sites used by *P. auritus* were identified in and around the Plashett Wood area, north of Lewes, East Sussex TQ 546110 115660 from a radio-tracking study (see Chapter 5), and were confirmed to be occupied by maternity colonies by emergence surveys Some of the roosts had previously been surveyed as part of a DPhil study on the comparison of the roost ecology of *P. auritus* and Serotine *Eptesicus serotinus* bats by Jessa Battersby in 1996 (Battersby, 1999). The 10 roosts sites (locations shown in Appendix A.3.1) were situated in and around Plashett Wood (Area 1). The minimum distance between two maternity roost sites was 20 metres and the maximum distance between any two maternity roosts was 4km.

In order to increase the number of roosts, and the geographic area in which the study was conducted, two additional areas were chosen to survey, Cowfold and the surrounding area in

West Sussex and the Ashdown Forest and the surrounding area in East Sussex. These areas were chosen because *P. auritus* roosts were previously identified (Battersby, 1999) in close proximity to one another and these could be accessed within a reasonable amount of time from the University of Sussex campus at Falmer. The three areas chosen for the study are shown in Appendix A.3.2.

Ten roosts were initially chosen in each of the additional areas but upon further survey it was found that some of the roost sites no longer had bats present and it also became apparent that it would not be feasible to record 30 roosts once per month throughout the season and it was therefore decided that five roosts from Area 2 in Ashdown Forest, East Sussex (locations shown in Appendix A.3.3) and five roosts from Area 3, in Cowfold, West Sussex (locations shown in Appendix A.3.4), in addition to the ten roosts in Area 1 would be studied.

3.2.2 - Preliminary roost inspections

Preliminary inspections were carried out at each of the roost sites in late April and early May 2007. These included an internal inspection to (i) ascertain whether *P. auritus* bats were present and where in the property the bats were roosting (if more than one roof space was present) and to; (ii) assess the number of bats present, their roosting positions within the building, and whether the roost was still likely to be functioning as a maternity roost (based on the number of droppings present and anecdotal information from the roost owners) also; (iii) carry out a preliminary dusk and dawn survey to identify the primary exit and entrance locations and finally; (iv) determine a suitable position for the recording equipment to be

situated for subsequent emergence surveys to obtain recordings of vocalisations from bats leaving and returning to their roost site.

3.2.3 - Emergence counts

Emergence counts were carried out on a monthly basis at all of 20 of the roost sites. A range of volunteer surveyors assisted with surveys from June to September including members of the local bat group, undergraduate and master's students from the University of Sussex, and individuals training for their NE bat licences. Volunteers have been used to record bats in monitoring programmes such as NMBP and roost surveys of species such as lesser horseshoes (Warren and Witter, 2002). Volunteers are potentially a valuable scientific resource providing manpower to conduct extensive surveys. Some scientists have questioned the reliability of data collected by inexperienced people (Darwall and Dulvy, 1996). However, other studies have found few differences in the data collected by experienced scientists and project volunteers. Standardisation and validation of the results collected by volunteers, however, is recommended (Silvertown, 2009).

With these considerations in mind, I accompanied each volunteer surveyor on the initial roost count and gave a brief training session prior to carrying out the roost count. Depending on their experience (and whether or not they were familiar with *P. auritus* calls on a bat detector) a recording of *P. auritus* echolocation and social calls was played, along with recordings of other house roosting bats that may be present, such as *Pipistrellus pipistrellus, P. pygmaeus* and *E. serotinus*. A field guide (Jones and Walsh, 2001) for identification of

bats in flight using bat detectors and flight patterns was provided, in addition to a bat detector (Batbox II Heterodyne) and a standardised recording sheet (shown in Appendix A.3.5).

I accompanied each volunteer surveyor at each roost site for their first survey and conducted an inter-observer reliability test comparing the number of bats emerging in each ten minute interval as recorded by myself and the volunteer surveyor, to ensure that our records of the numbers and species emerging were consistent. To maintain consistency each roost was assigned to one or two surveyors who then carried out all the counts at their roosts on a monthly basis. I set up the ultrasound recording equipment (the Ultrabat, see Chapter 2 for a description) at each roost site during the daytime and programmed it to commence recording 15 minutes before sunset and stop recording 15 minutes after sunrise. The Ultrabat recording device was placed adjacent to the primary roost exit and entrance (ascertained by the preliminary roost inspections) and the microphone of the Ultrabat was mounted on a 2 metre pole to enable recordings to be made of bats entering and exiting the roost, as shown in Figure 3.1. On occasion when either the equipment did not record accurately or the bats emerged from another exit (so the exact number that emerged was not determined or the equipment was situated in the wrong position) the count was repeated on a subsequent evening. Counts commenced 15 minutes before sunset and bats were counted at one minute intervals until at least 1.5 hours after sunset or until 15 minutes after the last bat had emerged. The direction of flight and other behavioural characteristics were noted as well as meteorological data such as temperature and humidity (recorded using Oregon Scientific WMR80 full wireless weather station) at the beginning and the end of the emergence survey and the light levels were recorded at 10 minutes intervals using a light meter (EasyView 30, Extech Instruments).

3.2.4 - Data analysis

Data were entered in Excel spreadsheets and analysed using the statistical package SPSS 17.0 for Windows.

3.3 - Results

3.3.1 - Emergence counts

Counts were obtained throughout the season from 20 *P. auritus* maternity roosts and these counts were used as a basis to estimate mean colony size for each roost across the season, as shown in Figure 3.2. The overall mean colony size was 25.8 bats (n = 20, s.e.m = 3.45). The smallest maximum count was 12 bats (August count, roost number 26, Green Lane Farm, Area 1). The largest maximum count was 98 bats (July count, roost number 9, Lantern Cottage, Area 2). Mean colony size before the first juveniles flew, was obtained by taking the maximum count for each roost during May and June, which was 25.9 bats (range = 8 – 81, s.e.m = 4.29). This increased to a mean of 32.9 bats (range 12 – 98, s.e.m = 4.63) when the juveniles became volant, obtained by taking the maximum count for each roost during July and August. There was therefore a mean increase of 30.55% in colony size, in 2007.

3.3.2 - Emergence behaviour

For all 20 *P. auritus* roosts, the mean time for emergence of the first bat was 21.9 minutes after sunset (s.e.m = 0.76, n = 100, range = 9 - 36), and the mean median bat emergence was 34.5 minutes after sunset (s.e.m = 0.67, n = 100, range = 16 – 53). A typical emergence

pattern was for 1 to 3 bats to emerge approximately 15 minutes before the main emergence. Bats would subsequently emerge in groups of 2 – 5 and typically flew directly to cover, such as hedgerows or trees in the vicinity of the roost. Median emergence time was significantly correlated with the time of sunset ($r^2 = 0.97$, df = 98 $p < 0.0001$, Figure 3.3), whilst none of temperature ($r^2 = 0.02$, df = 98, $p = 0.089$), humidity ($r^2 = 0.002$, df = 98, $p = 0.891$) or light intensity (lux) ($r^2 = 0.012$, df = 98, $p = 0.250$) showed any significant correlation with median emergence time.

The mean duration of emergence was 34.9 minutes (s.e.m = 1.10, n = 100, range = 11 – 58. Duration of emergence was significantly correlated with the number of bats emerging from the roost ($r^2 = 0.602$, df = 98, Figure 3.4). The relationship between rainfall and emergence time could not be tested for statistically because on very rainy nights the survey was abandoned (to avoid potential damage to equipment), and persistent rainfall (i.e. for at least 50% of the survey period) only occurred on six of the 100 nights that the emergence surveys were carried out (to prevent excessive rain potentially damaging recording equipment). Bats emerged as normal on all six nights.

3.3.3 - Social calls recorded at roost sites: seasonal patterns.

A total of 11,484 *P. auritus* social calls were recorded at the 20 maternity roost sites from May 2007 to September 2007. There were significant differences between months in the mean number of calls recorded at maternity roost sites (Kruskal-Wallis ANOVA H = 23.52, d.f = 4, $p < 0.001$; as shown in Figure 3.5). A total of 10 post-hoc pair-wise comparisons was undertaken (using Mann-Whitney U with a Bonferroni correction to reduce Type 1 error rate (.05/5) resulting in a critical value for significance of 0.01) to investigate where the

differences were significant. Figure 3.5 details the results of the differences in the mean number of calls per month. There were significantly more social calls recorded at maternity roost sites later in the season (September) compared to earlier in the season (May to June).

One possibility is that the increased number of social calls recorded later in the season may be a result of the longer recording time (as a consequence of fewer daylight hours). For example, the recording time from sunset to sunrise for roost number six was 445 minutes in June compared to 702 minutes in September. Therefore, the mean number of social calls per hour was calculated for each roost and there were significant differences between months in the social call rate per hour (Kruskal-Wallis ANOVA H = 10.47, d.f = 4, p = 0.03; as shown in Figure 3.6). A total of 10 post-hoc pair-wise comparisons was undertaken (using Mann-Whitney U with a Bonferroni correction to reduce Type 1 error rate (.05/5) resulting in a critical value for significance of 0.01) to investigate where the differences were significant. Figure 3.6 details the results of the differences in the monthly mean number of calls per hour. There were significantly more social calls per hour recorded at maternity roost sites in September compared to June (but not May).

The number of social calls showed a linear increase from June to September, whereas, the number of bats emerging decreased sharply from August to September, as shown in Figure 3.7. A Scheirer-Ray-Hare test showed that there were significant differences in the number of social calls recorded at maternity roost sites in relation to month and the number of bats emerging but there was no evidence of an interaction between month and number of bats emerging (Table 3.1). Nonetheless, the mean overall number of social calls recorded at each

roost site from May to September 2007 was positively correlated with the mean colony size, (Spearman rank correlation r_s =0.932, n = 20, p < 0.01) as shown in Figure 3.8.

3.3.4 - Temporal patterns of social call production

To examine whether there were temporal differences in the production of social calls at *P. auritus* maternity roosts, the number of calls emitted per quarter of night was analysed. The total length of time was calculated from dusk to dawn, for each night's recording, and it was divided into quarters. Each social call was then allocated to a specific time quarter from Q1 to Q4 and the social call rate per hour was subsequently calculated for each quarter. There was evidence of a difference in the mean number of social calls per hour on a quarterly basis per night, but no evidence of monthly differences or evidence of an interaction between month and quarterly night period, as determined by the results of the Scheirer-Ray-Hare test shown in Table 3.2 and Figure 3.9. Furthermore, the seasonal increase in the calling rate occurs in Q4. A Kruskal-Wallis test also found that there were significant differences between the social calls per hour analysed in quarterly periods (Kruskal-Wallis ANOVA 163.50, df = 5, p < 0.001). Post-hoc Mann-Whitney U tests were subsequently applied to investigate where the differences in temporal calling were significant. There were significantly more social calls per hour recorded in Q4 than in Q1, Q2 and Q3, significantly more calls recorded per hour in Q1 than in Q2 and Q3, and no significant differences between the number of social calls recorded in Q2 and Q3 as shown in Figure 3.10.

3.3.5 - Seasonal patterning of specific call types

In Chapter 2 the 11,484 calls recorded were classified into three types Type A, B and C, based on their call structure. The majority of calls (96.6% n = 11,101) were classified as Type A calls, and the cluster analysis indicated that these could be further subdivided on the basis of their acoustic parameters into six different call types (Calls 1 to 6, Type B and C were renamed Call 7 and Call 8 respectively). Using the data from the k-means cluster analysis (see Chapter 2 section 2.3.6), a significant association was found between call type and the month in with the call was produced (χ^2 = 1615, d.f 5, p > 0.001). However, this association test combined all calls and all months and did not provide any information as to which calls, if any, were more frequently associated with a particular month. Therefore, post-hoc analysis was carried out using cross-tabulation and converting standard residuals (the difference between observed and expected values) to a 'Z' score. This, in turn, was compared to a critical value for alpha using a conservative critical value of Z = +/- 3.15, (corresponding to a p value of 0.0016, applying a Bonferroni correction of .05/30 for multiple comparisons). Table 3.3 shows the results of the cross-tabulation for the χ^2 test of association.

Six positive and four negative associations were significantly greater than the critical value of Z = +/- 3.15, p = 0.0013. Call 1 and Call 5 were significantly associated with the month of June, Call 3 and Call 4 were significantly associated with the month of May, Call 2 was significantly associated with the month of September, and Call 6 was significantly associated with the month of August. By contrast, Call 2 was less frequently associated with the month of May, Call 3 and Call 6 were less frequently associated with the month of September, and Call 4 was less frequently associated with the month of June. However, as there was an overall increase in the number of social calls recorded in August and September, the number

of calls assigned to each cluster type was analysed as a proportion of the overall call database, for each month, as shown in Figure 3.11. Most of the calls did not exhibit a great deal of seasonal variation. Call 2, which is differentiated from the other call types by having a longer duration and lower frequency of maximum energy (see Chapter 2 section 2.3.5), showed an increase in occurrence from May to July, a small decrease in August followed by a sharp increase in September. Call 3 occurred at a higher frequency in May compared to the rest of the season. Call 7 and 8 comprised a very small proportion overall, 1.87% (n = 215) and 1.46% (n = 168) respectively, of the total number of social calls recorded at roost sites, and were therefore, analysed separately. The number of calls assigned to each call type was analysed as a proportion of the overall call database for each month. There was a significant difference between months (Kruskal –Wallis Anova H = 16.176, d.f. = 4, p = 0.003) with the majority of calls for both calls types recorded more frequently later in the season, as shown in Figure 3.12.

3.3.6 - Temporal patterns of specific call types

In order to investigate whether the six different Type A calls were more frequently associated with occurrence at a given time of night (i.e. when bats are leaving and/or returning to the roost, or when females returned to feed their offspring), the analysis was carried out using cross-tabulation and converting standard residuals, the difference between observed and expected values, to a 'Z' score which, in turn, were compared to a critical value for alpha. Using a conservative critical value of Z = +/- 3.09 (corresponding to a p value of 0.002, derived from a Bonferroni correction of .05/24 for multiple comparisons), Table 3.4 shows the results of the cross-tabulation for the Chi Sq. test of association. There were no calls associated with occurrence for each quarter of a nights' recording. The proportions of calls

for each quarter, shown by the bar charts in Figure 3.13, also reflect that all of the call types were equally likely to occur in similar proportions for each quarter of a nights recording. There were also no significant differences between quarters for call 7 (Kruskal-Wallis Anoca H = 4.691. d.f. = 3, p = 0.196) and call 8 (Kruskal –Wallis Anova H = 6.646, d.f. = 3, p = 0.084).

3.3.7 - Testing for roost and location call types

The six Type A calls occurred at all roost sites (although in different proportions see Figure 3.14). Type B (call 7) was recorded at 18 roost locations and Type C calls (call 8) was recorded at 15 roost locations. This suggests that the call types are likely to be species-specific as opposed to roost or location specific. However, the potential for further analysis of within call type for both roost and geographic areas are discussed in section 3.4.

3.4 - Discussion

The *P. auritus* maternity roosts surveyed in this study varied in size from as few as 12 bats up to 98, and the number of social calls recorded at these roost sites was highly correlated with the numbers of bats present in the colony. This correlation suggests that most individuals at maternity roosts may contribute to the vocalisations as opposed to a subset of dominant individuals repeatedly calling. *P. auritus* live in stable colonies comprised of matrilineally related females (Burland et al., 1999; Veith et al., 2004) with little immigration or emigration (Entwistle, 1994; Entwistle et al., 2000) and are long-lived. It is possible, therefore, that one function of social calling at *P. auritus* maternity roosts is to maintain and strengthen social bonds amongst colony members.

Social calls at roosts occur in the dawn swarming period (Briggs and King, 1998) and this study found that there were significantly more *P. auritus* social calls in the last quarter of the night prior to re-entry to the roost. The function of dawn swarming is not known for certain and several hypotheses have been put forward but none have been tested. These hypotheses include, for example, information transfer about good foraging areas that night, teaching the young to fly, and anti-predatory behaviour. However, calls may also be given periodically at roosting sites to attract conspecifics or guide colony members to potential roosts. A study by Schöner et al., (2010) demonstrated that simulated social calls attracted bats to roost sites and the bats could discriminate between the synthesised social calls of conspecifics and the calls of other sympatric species. Schöner et al., (2010) played back simulated Bechstein's bat *Myotis bechsteinii* and simulated Natterer's bat *M. nattereri* in an area of deciduous forest in Germany where *M. bechsteinii, M. nattereri*, and *P. auritus* colonies were present in an area of approximately 0.5km^2. They placed the speakers of the Sussex Autobat (Hill and Greenaway, 2005) inside bat boxes (without blocking the entrances) and subsequently emitted simulated *M. bechsteinii* and *M. nattereri* socials calls monitoring the responses using a combination of infra-red video recording and passive integrated transponder (PIT) tags. *M. bechsteinii, M. nattereri* and *P. auritus* approached the experimental bat boxes significantly more often when bat social calls were played back compared to control nights without calls. *M. bechsteinii* and *M. nattereri* approached the speakers broadcasting the call significantly more often when the calls of conspecifics had been played compared of the respective other species. Approaching *P. auritus* did not discriminate between *M. bechsteinii* and *M. nattereri* (Schöner et al., 2010)

An alternative hypothesis for the function of calls at roost sites would be that the calls serve to advertise the new roosting locations to conspecifics. A study by Chaverri et al, (2010)

showed that Spix's disk-winged bats *Thyroptera tricolor*, which had been captured and subsequently released close to an unfamiliar roost in which a conspecific had been placed, were attracted to the roost by social calls of this conspecific. Female *P. auritus* have been shown to switch roosts during the summer, particularly females utilising tree roosts (Murphy et al., in prep), and social calls may function to advertise new roosting locations to colony members. However, female *P. auritus* roosting in buildings show less evidence of roost switching behaviour (Entwistle, 1994; Entwistle et al., 2000; Murphy et al., in prep) but they do regularly switch roosting positions within buildings (Battersby 1999).

Animals may learn to recognise individually distinct calls of group mates. One method for advertising group membership is to adopt a common vocalisation (Wilkinson, 1995; Boughman, 1997; Boughman and Wilkinson, 1998; Frommolt et al., 2003). The greater spear-nosed bat *Phyllostomus hastatus* emits audible screech calls when departing from roosts that attract group members to foraging sites (Boughman, 1997; Wilkinson and Boughman, 1998). Group members could discriminate between calls of their groups members and calls given by other bats that were not members of their group (Boughman and Wilkinson, 1998). Analysis of acoustic variables of the screech calls given by *P. hastaus* found group differences but not individual differences in call structure (Boughman and Wilkinson, 1998). This study did not find any evidence that *P. auritus* colonies used different call types that could allow for discrimination between members of adjacent colonies or populations as all the call types were recorded at each of the roosts but there may have been differences within call type.

The increase in the number of social calls in September, despite the decrease in mean colony size, suggest that either all remaining bats are calling more frequently or individuals in the

vicinity of the roost site may be vocalising repeatedly or potentially both scenarios could be a contributing factor. In September, maternity colonies begin to disband as bats disperse to transient roosts prior to hibernation (Heise and Schmidt, 1988). There is some evidence that mating may occur in maternity roosts in *P. auritus* (Swift, 1998) and, as the mating process begins in autumn, it could be that the increase in social calls could be as a function of the onset of the mating process. Furmankiewicz (2004) reported that only sequences of FM calls (typical of type A) were recorded when bats were emerging or entering the summer maternity roost site and that the calls differed from the social calls recorded at swarming sites, which were more diverse in structure. However, Furmankiewicz (2004) only recorded 118 social calls at one maternity roost site in September. This study recorded 3626 social calls across 20 maternity roosts in September and found a low number of calls (approximately 8.14%) that differed in structure to the six Type A calls. These calls were categorised as Call 7 (Type B Chapter 2) and Call 8 (Type C Chapter 2). These calls were similar in structure to the undulating (Call 7) and V call (Call 8) described by Furmankiewicz (2004) at underground swarming sites in Poland.

Swarming at hibernation sites has been reported for *P. auritus* in continental Europe (Veith et al., 2004; Furmankiewicz and Altringham, 2007) and these studies have implied that the function of swarming may be related to mating and gene flow. However, studies on swarming at hibernation sites in the U.K. have found less evidence for *P. auritus* swarming behaviour (Greenaway, F. pers.comm) compared to other species such as Natterer's *Myotis nattereri* and Daubenton's bat *Myotis daubentonii* (Parsons et al., 2003) and, also, that the majority of *P. auritus* recorded at these sites were juveniles (Greenaway, F. pers.comm). Entwistle (1994) concluded that the likely mating system in *P. auritus* is a random, promiscuous one in which swarming occurs and females mate with many males, including mating by males with

torpid females at hibernation sites and mating in early spring. It is, therefore, possible that the increase in social calling at maternity roost sites in the UK in September may function in the mating process for males to advertise their presence to females at maternity roosts. Call type two, which was longer in duration with a lower frequency of maximum energy, occurred more frequently in September as did calls seven and eight, which were more diverse in call structure compared to the typical Type A calls that were recorded throughout the season.

Acoustic variation has been shown to occur in animal vocalisations at many different levels not only amongst group members. Most of the studies on variation in animal vocalisations have been carried out on birds. For example, regional variation or dialects have been demonstrated in the vocalisations of Black capped chickadee *Poecile atricapillus*. Upon analysis of gargle types, 88.7% were found unique to individual populations rather than shared among or between populations (Miyasato and Baker, 1999). A study of regional variation of the social calls of Kuhl's Pipistrelle *Pipistrellus kuhlii* found evidence that the peak frequencies of two adjacent populations differed (Russo and Jones, 1999). However, a study on the regional variation of complex bat songs in male Brazilian free-tailed bats *Tararida brasiliensis* did not find evidence of variation of song types or diversity between differing locations (Bohn et al., 2009). This study found that all of call types occurred at most of the roosts and all of the regions, but this study did not analyse within each call type to see if there were differences in the acoustic parameters at a finer scale. For example, differences between peak frequencies or maximum frequencies between adjacent colonies and / or aeras. However, this type of study may be better conducted under more controlled conditions where the identity of the individual calling is known and more precise measurements of the acoustic parameters of the social calls can be made. Furthermore, calls from captive individuals from known colonies could be used to test hypotheses with respect to call function, as well as

whether roost mates can discriminate roost members from intruders. These types of questions could be addressed by using playback techniques in controlled conditions.

3.5 – Summary

- Mean colony size was positively correlated with the number of social calls recorded.
- There were significantly more social calls recorded at maternity roosts later in the season (September) compared to earlier in the season (May to June). This is in contrast to the number of bats recorded emerging at roost sites in September.
- Temporal analysis of call production indicated that there were significantly more calls recorded in the time period (quarter of night) prior to dawn.
- Most of the call types described (Chapter 2) did not exhibit a great deal of seasonal variation but Call 2 (see Chapter 2 section 2.3.5), which is differentiated from other call types by having a longer duration and lower frequency of maximum energy, exhibited a sharp increase in September. Call 3 occurred at a higher frequency in May compared to the rest of the season.
- There was no evidence of calls being associated with occurrence for a given time of night (i.e. departing and /or returning to roost sites).

Figure 3.1: Ultrabat recording equipment placed outside roost B2 Sleeches Farmhouse. Number one denotes the primary exit location (from under the lead flashing adjacent to the rear chimney stack). Number two denotes the silver microphone box mounted on a pole to record bat vocalisations as the bats enter and exit the roost. Number three denotes the recording equipment placed inside a waterproof casing.

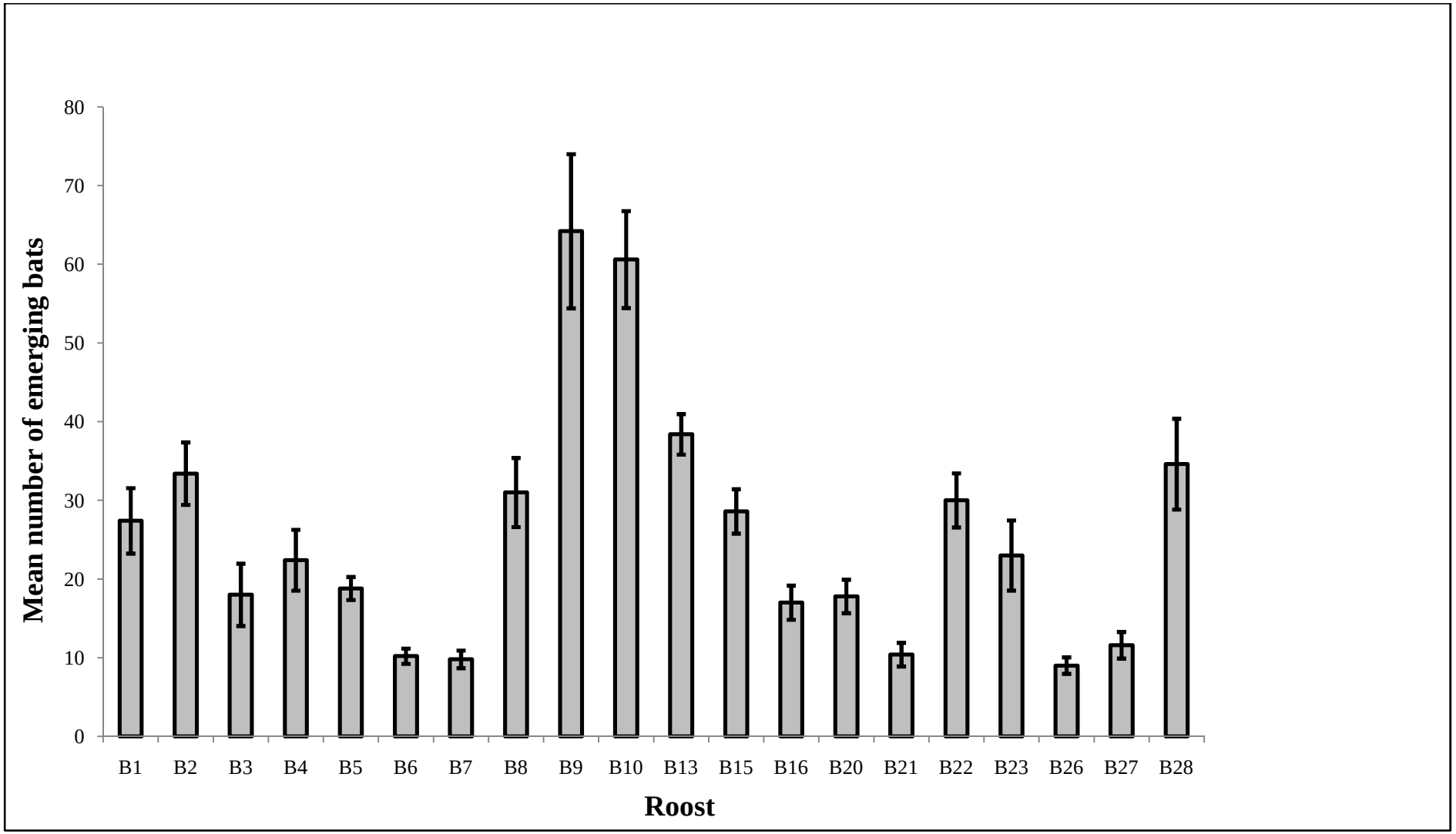

Figure 3.2: Mean emergence counts for each of the 20 *P. auritus* maternity roosts, obtained from monthly emergence survey counts, from May to September 2007. The error bars show the standard error of the mean.

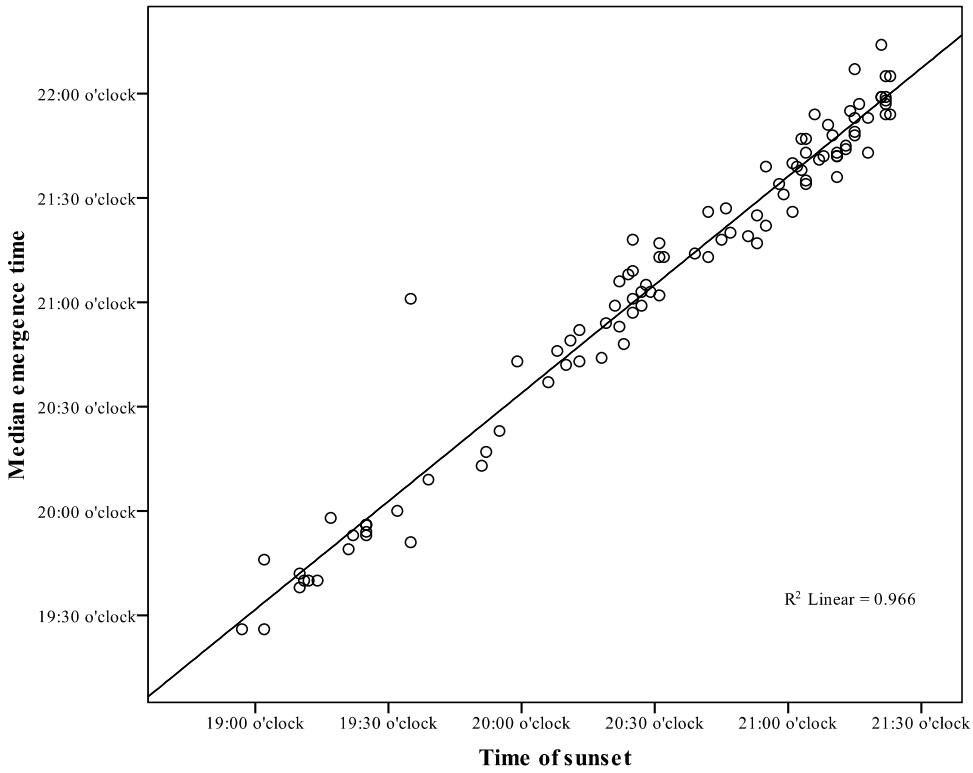

Figure 3.3: Correlation between the time of sunset and median bat emergence time.

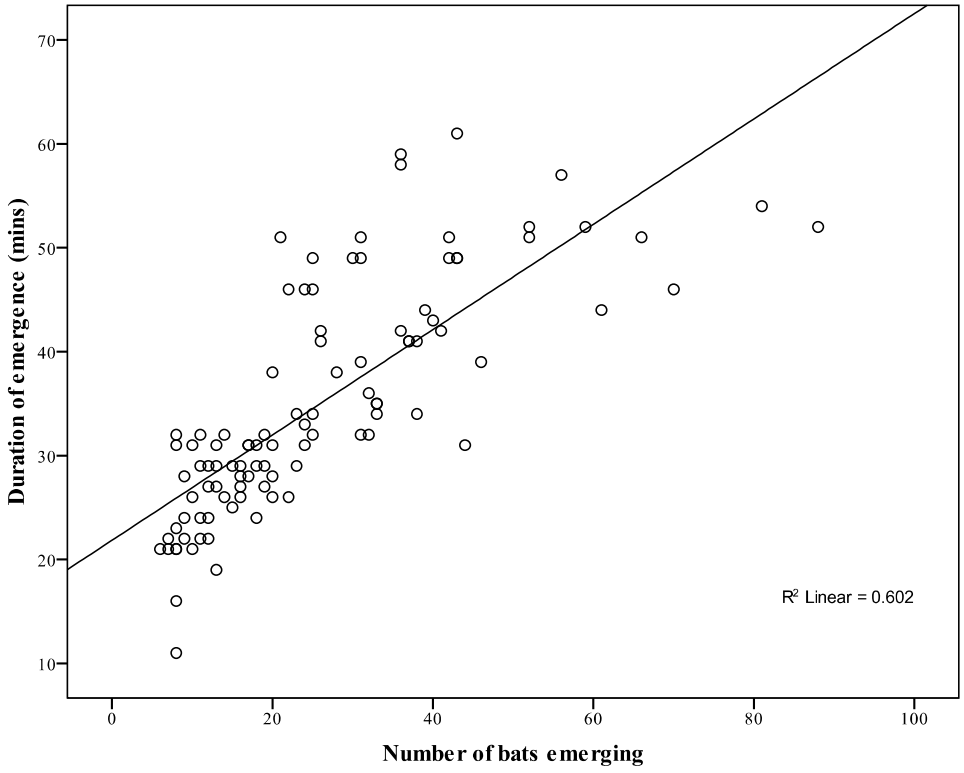

Figure 3.4: Correlation between the duration of the emergence and the number of bats emerging.

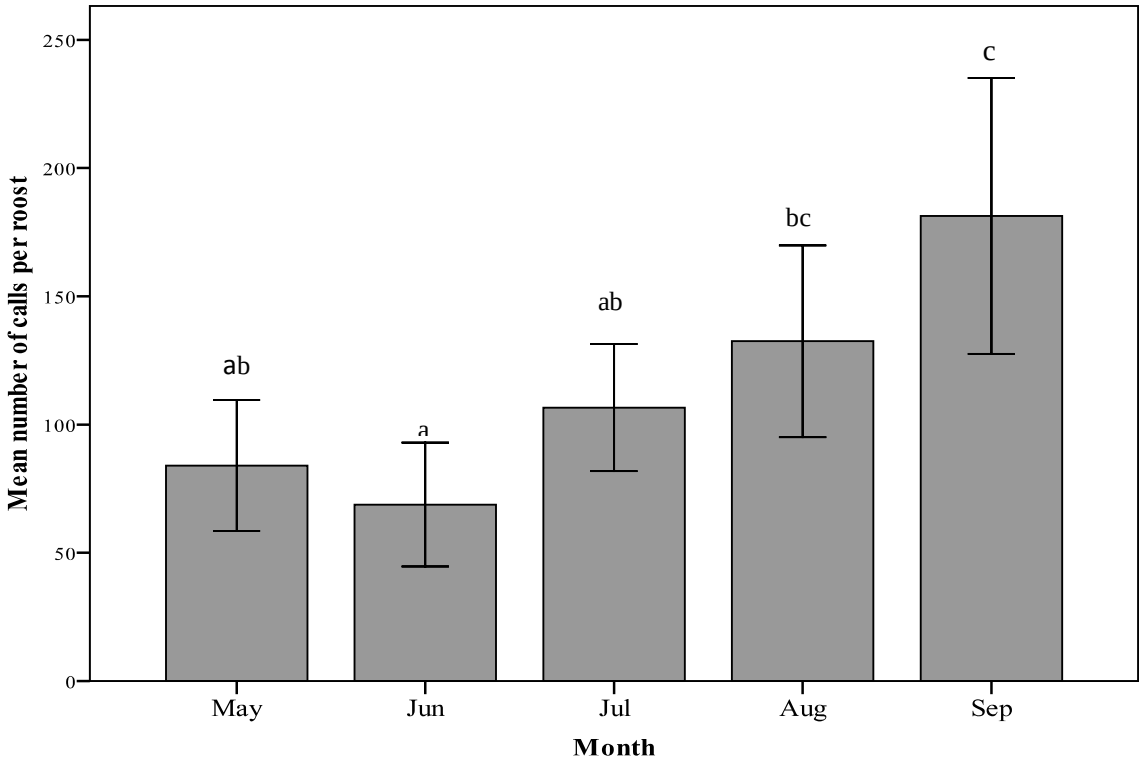

Figure 3.5: Mean monthly number of social calls recorded at twenty maternity roost sites in Sussex from May to September 2007. The error bars show the standard error of the mean. There was a significant difference in the monthly number of calls. Where the letters are different it indicates a significant difference between group means.

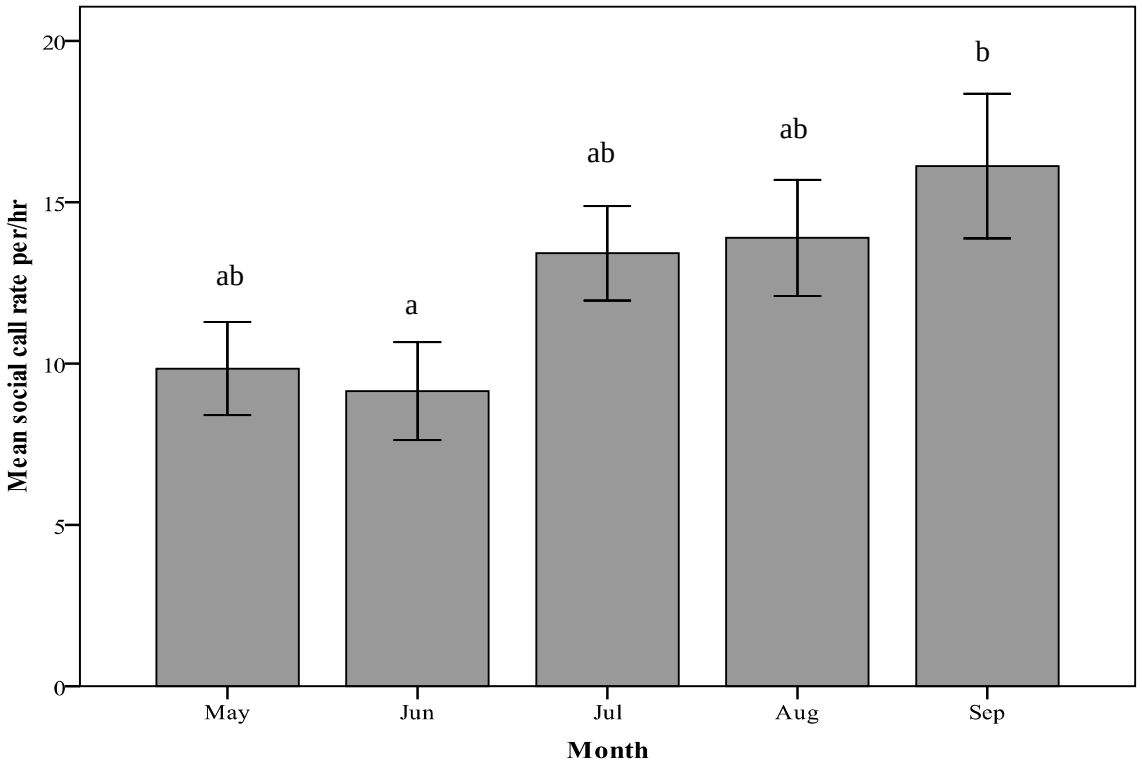

Figure 3.6: Mean number of social calls per hour recorded at twenty maternity roost sites in Sussex from May to September 2007. The error bars show the standard error of the mean. There was a significant difference in the monthly mean social call rate. Where the letters are different it indicates a significant difference between group means.

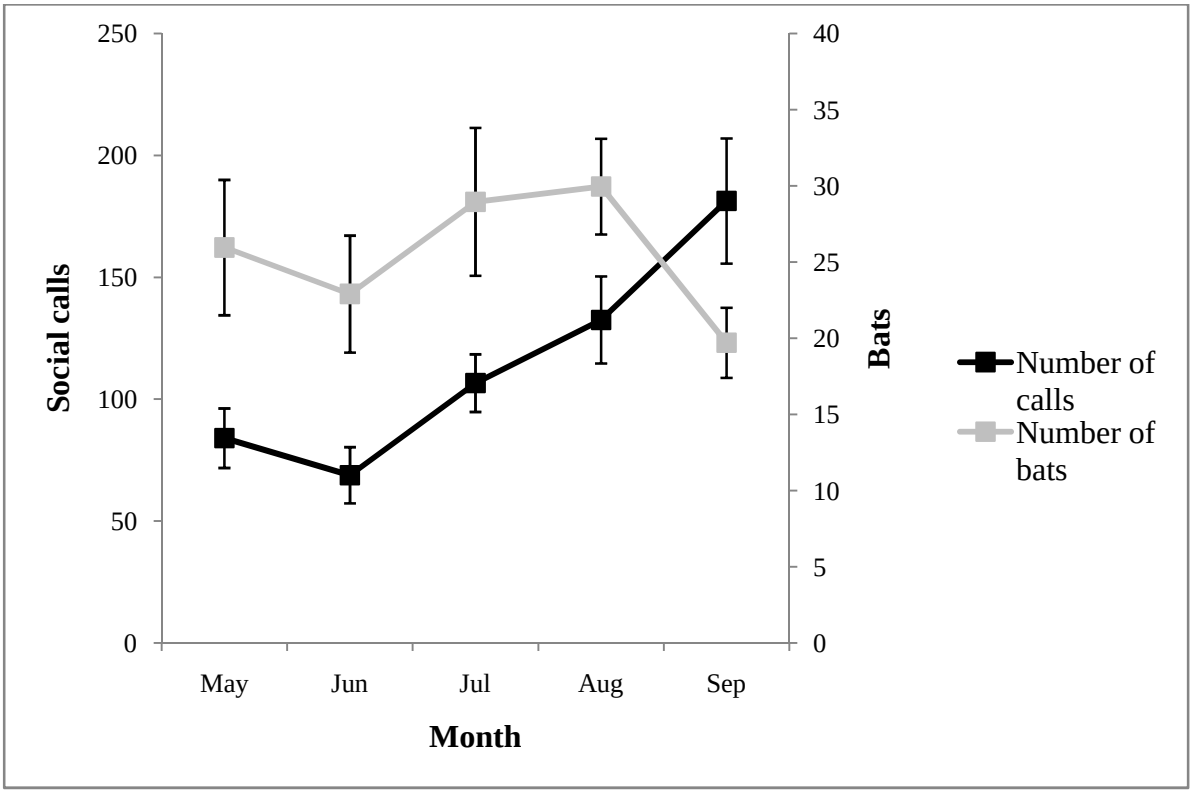

Figure 3.7: Relationship between the mean number of bats emerging and the mean number of social calls in each of five months. The error bats show the standard error of the mean.

Source of variation	**SS**	**SS/MS total**	**d.f.**	***P*-value**
Month	52483.34	26.20	4	< 0.001
Number of bats emerging	425180.58	212.27	43	< 0.001
Month*Number of bats	63768.46	31.83	33	0.525

Table 3.1: Results of the Scheirer-Ray-Hare test on the number of social calls recorded at maternity roost sites in relation to month and the number of bats emerging.

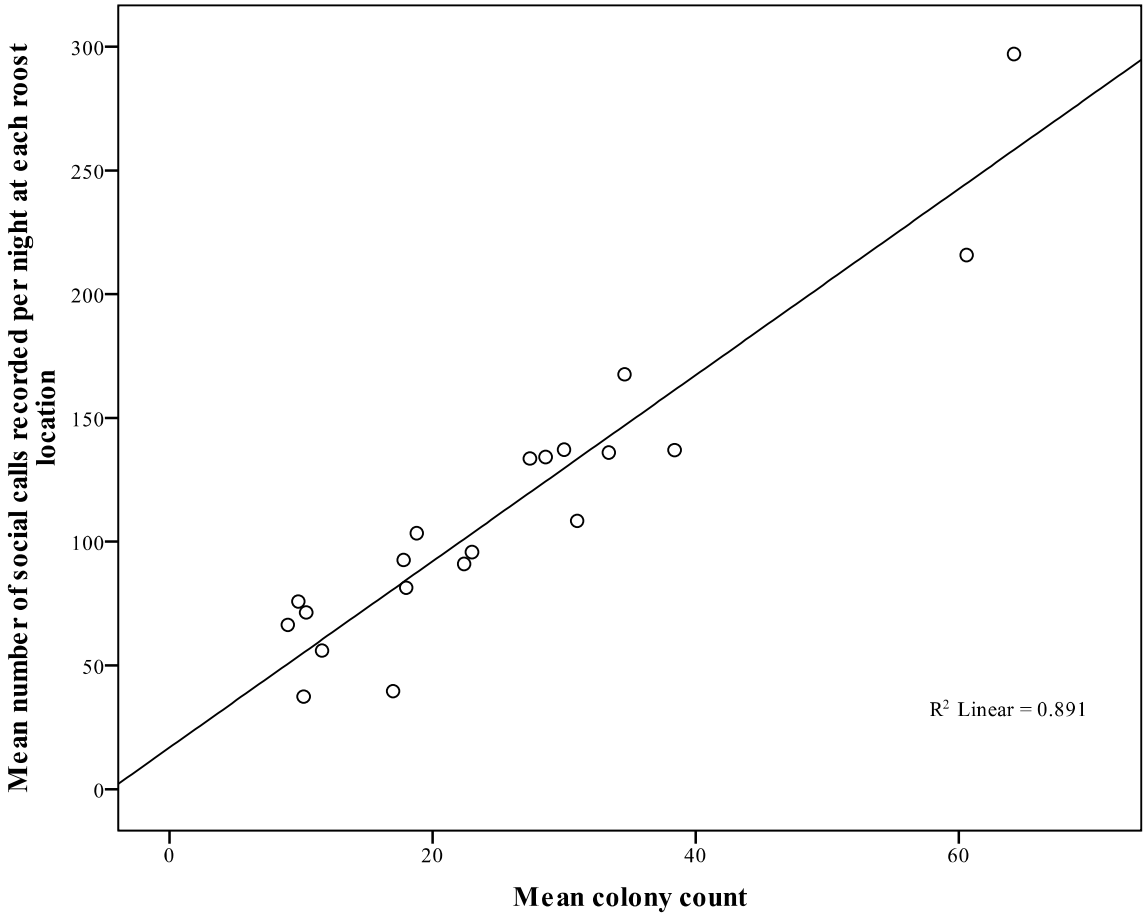

Figure 3.8: Correlation between the mean number of social calls recorded and the mean colony count.

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Source of variation	SS	SS/MS total	d.f.	*P*-value
Month	1994	0.315	4	0.988
Quarter	57176	8.99	3	0.029
Month*Quarter interation	2242	63.28	24	1.000

Table 3.2: Results of the Scheirer-Ray-Hare test on the number of social calls recorded at maternity roost sites in relation to the quarterly night period and the month in which the call was recorded.

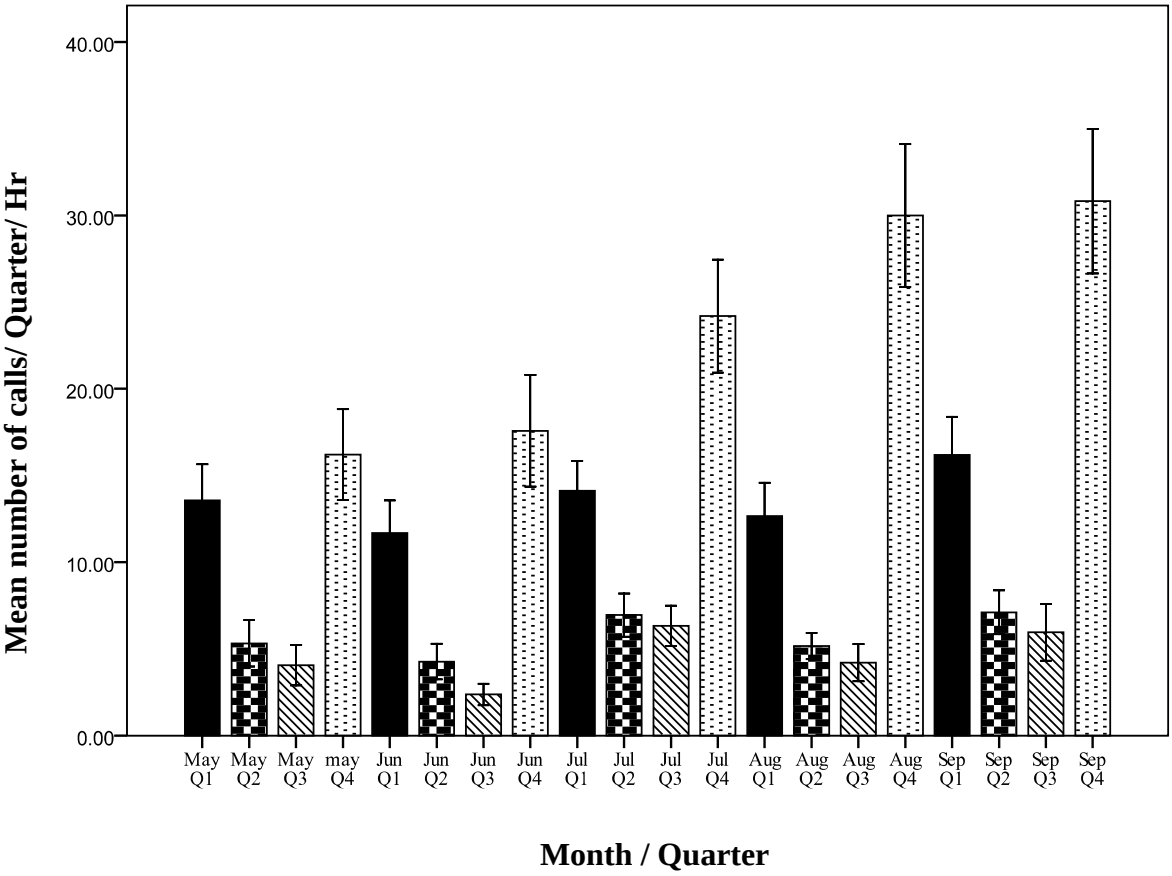


Figure 3.9: Mean number of *P. auritus* social calls per hour, per quarterly night time recording period for each month. The error bars show the standard error of the mean.

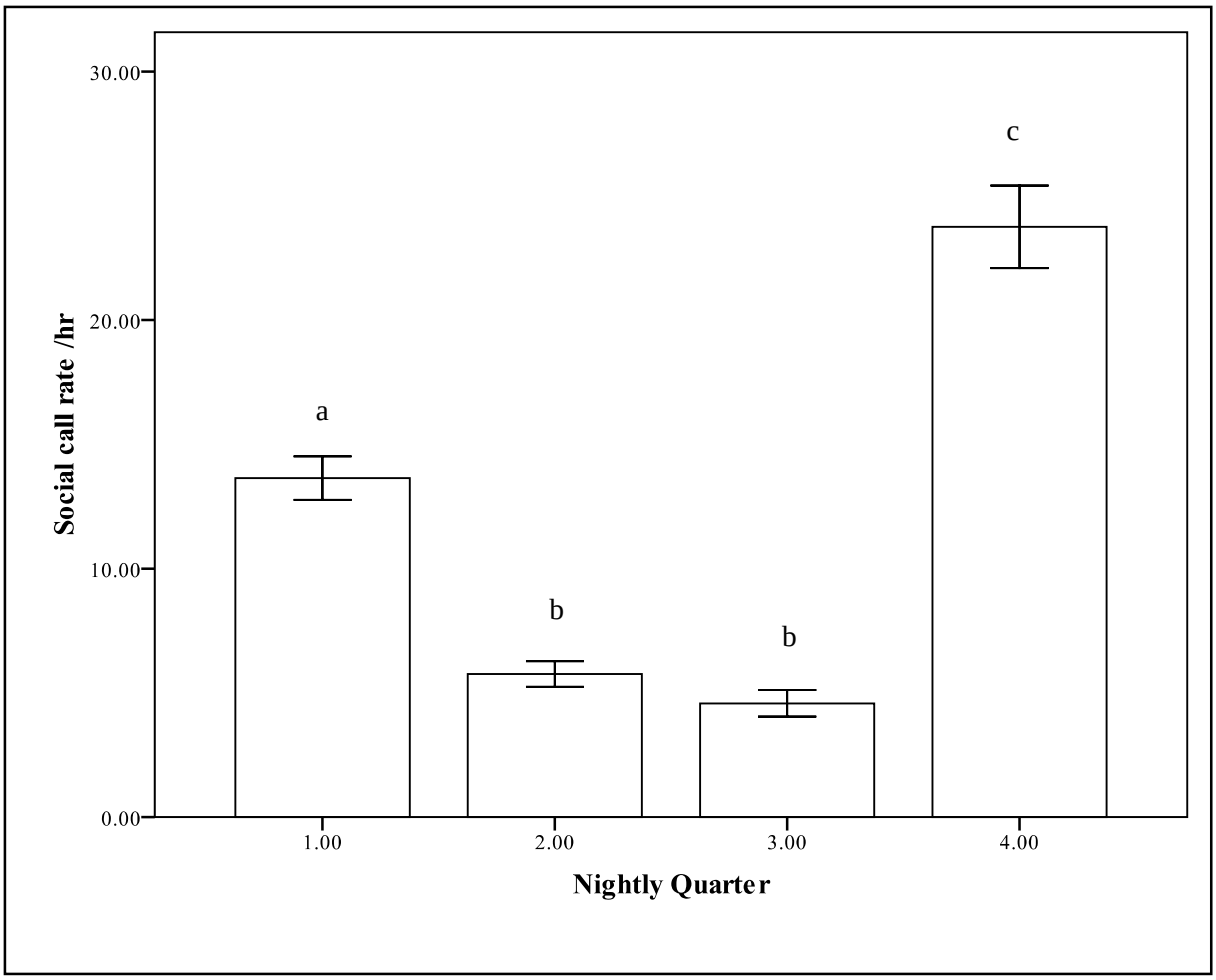


Figure 3.10: Temporal variation in the number of social calls recorded. Where the letters are different it indicates significant differences between groups.

CALLTYPE * MONTH Crosstabulation

			MONTH					
			MAY	JUN	JUL	AUG	SEP	Total
CALLTYP	1	Count	349	399	521	578	743	2590
		Expected Count	389.9	318.2	484.4	591.7	805.9	2590.0
		Std. Residual	-2.1	**4.5**	1.7	-.6	-2.2	
	2	Count	182	227	454	499	1038	2400
		Expected Count	361.3	294.9	448.8	548.3	746.7	2400.0
		Std. Residual	**-9.4**	**-4.0**	.2	-2.1	**10.7**	
	3	Count	260	69	112	156	181	778
		Expected Count	117.1	95.6	145.5	177.7	242.1	778.0
		Std. Residual	**13.2**	-2.7	-2.8	-1.6	**-3.9**	
	4	Count	353	136	287	343	406	1525
		Expected Count	229.6	187.4	285.2	348.4	474.5	1525.0
		Std. Residual	**8.1**	**-3.8**	.1	-.3	-3.1	
	5	Count	170	214	196	272	390	1242
		Expected Count	187.0	152.6	232.3	283.7	386.4	1242.0
		Std. Residual	-1.2	**5.0**	-2.4	-.7	.2	
	6	Count	357	319	506	688	696	2566
		Expected Count	386.3	315.3	479.9	586.2	798.4	2566.0
		Std. Residual	-1.5	.2	1.2	**4.2**	**-3.6**	
Total		Count	1671	1364	2076	2536	3454	11101
		Expected Count	1671.0	1364.0	2076.0	2536.0	3454.0	11101.0

Table 3.3: Results of Chi Sq test of association. Standardised residuals that have a positive value mean that the cell was over-represented in the actual sample, compared to the expected frequency. Using a critical value of Z =+/- 3.15, there are five positive (shown in blue font) and five negative (shown in red font) associations between call type and the month that the call was produced.

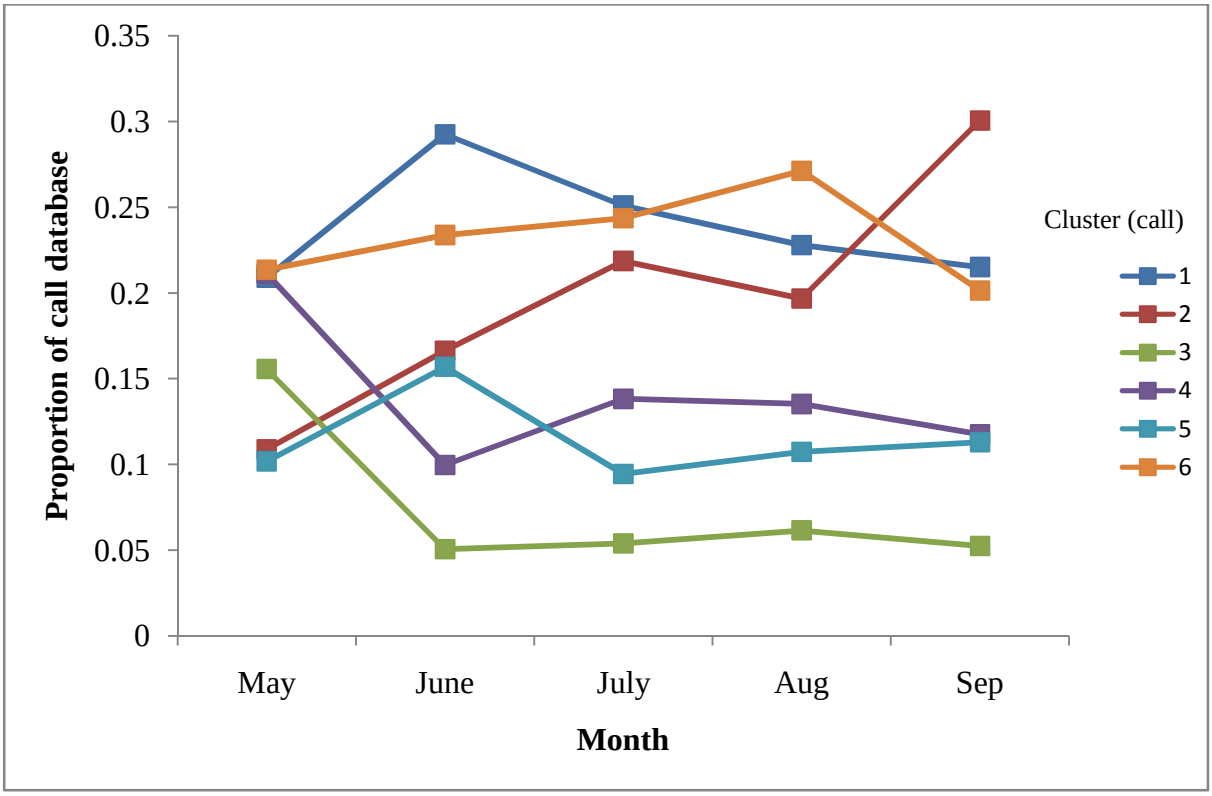


Figure 3.11: The proportion of each of the six call types (clusters) for 'Type A' calls recorded at maternity roost sites from May to September 2007.

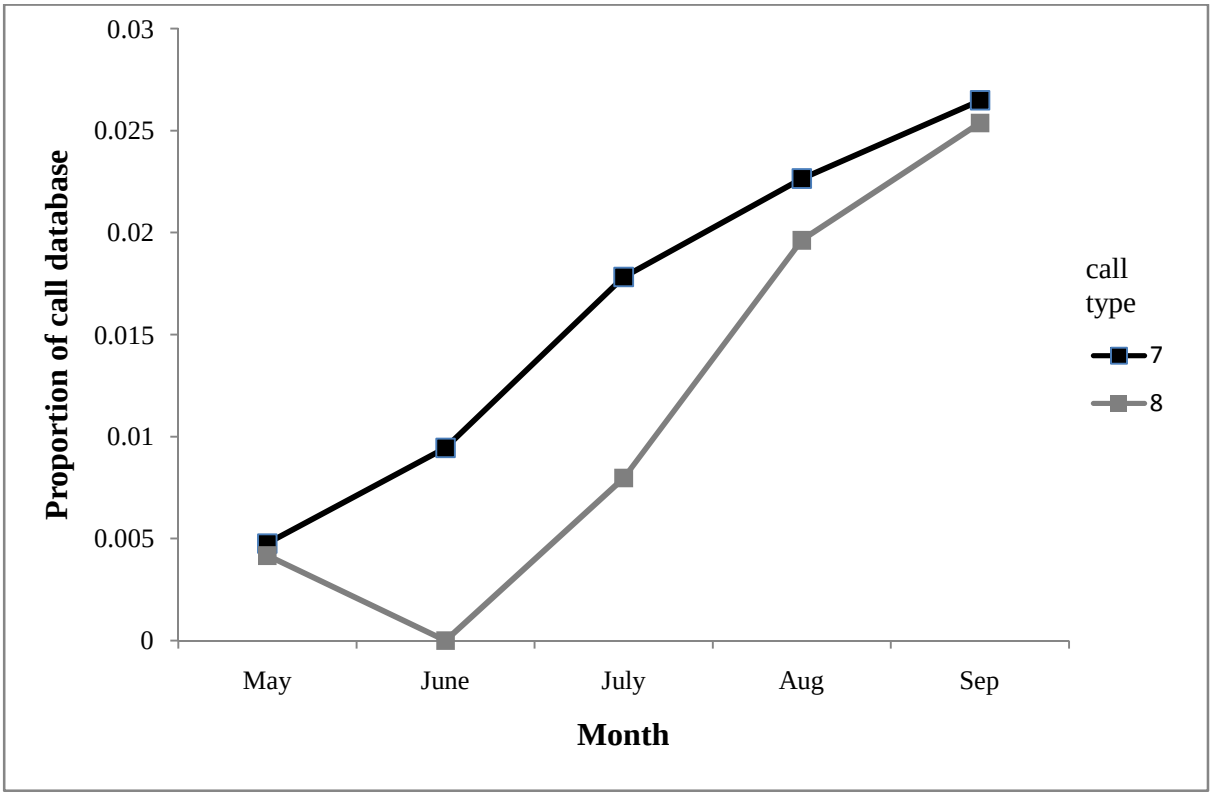


Figure 3.12: The proportion of call type 7 and 8 recorded at maternity roost sites from May to September 2007.

CALLTYPE * QUARTER Crosstabulation

			QUARTER				
			1	2	3	4	Total
CALLTYPE	1	Count	751	289	247	1303	2590
		Expected Count	682.4	326.4	257.3	1323.8	2590.0
		Std. Residual	2.6	-2.1	-.6	-.6	
	2	Count	530	341	227	1302	2400
		Expected Count	632.4	302.5	238.5	1226.7	2400.0
		Std. Residual	-3.0	2.2	-.7	2.1	
	3	Count	268	80	62	368	778
		Expected Count	205.0	98.0	77.3	397.7	778.0
		Std. Residual	2.4	-1.8	-1.7	-1.5	
	4	Count	418	231	179	697	1525
		Expected Count	401.8	192.2	151.5	779.5	1525.0
		Std. Residual	.8	2.8	2.2	-3.0	
	5	Count	279	158	131	674	1242
		Expected Count	327.3	156.5	123.4	634.8	1242.0
		Std. Residual	-2.7	.1	.7	1.6	
	6	Count	679	300	257	1330	2566
		Expected Count	676.1	323.4	255.0	1311.5	2566.0
		Std. Residual	.1	-1.3	.1	.5	
Total		Count	2925	1399	1103	5674	11101
		Expected Count	2925.0	1399.0	1103.0	5674.0	11101.0

Table 3.4: Results of Chi Sq test of association. Standardised residuals that have a positive value mean that the cell was over-represented in the actual sample, compared to the expected frequency. There were no positive or negative associations that reached the critical value of Z = +/- 3.09.

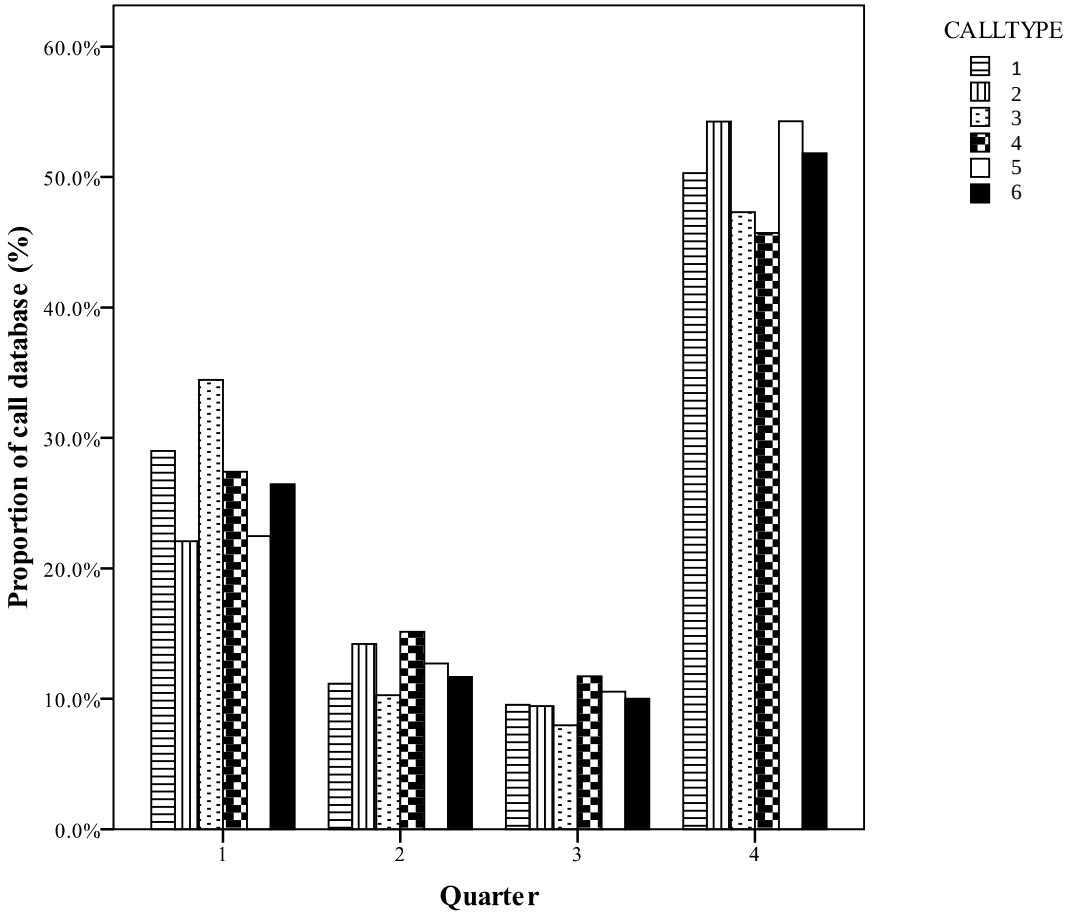


Figure 3.13: The proportion of all Type A calls that each of the six call types accounted for in each quarter.

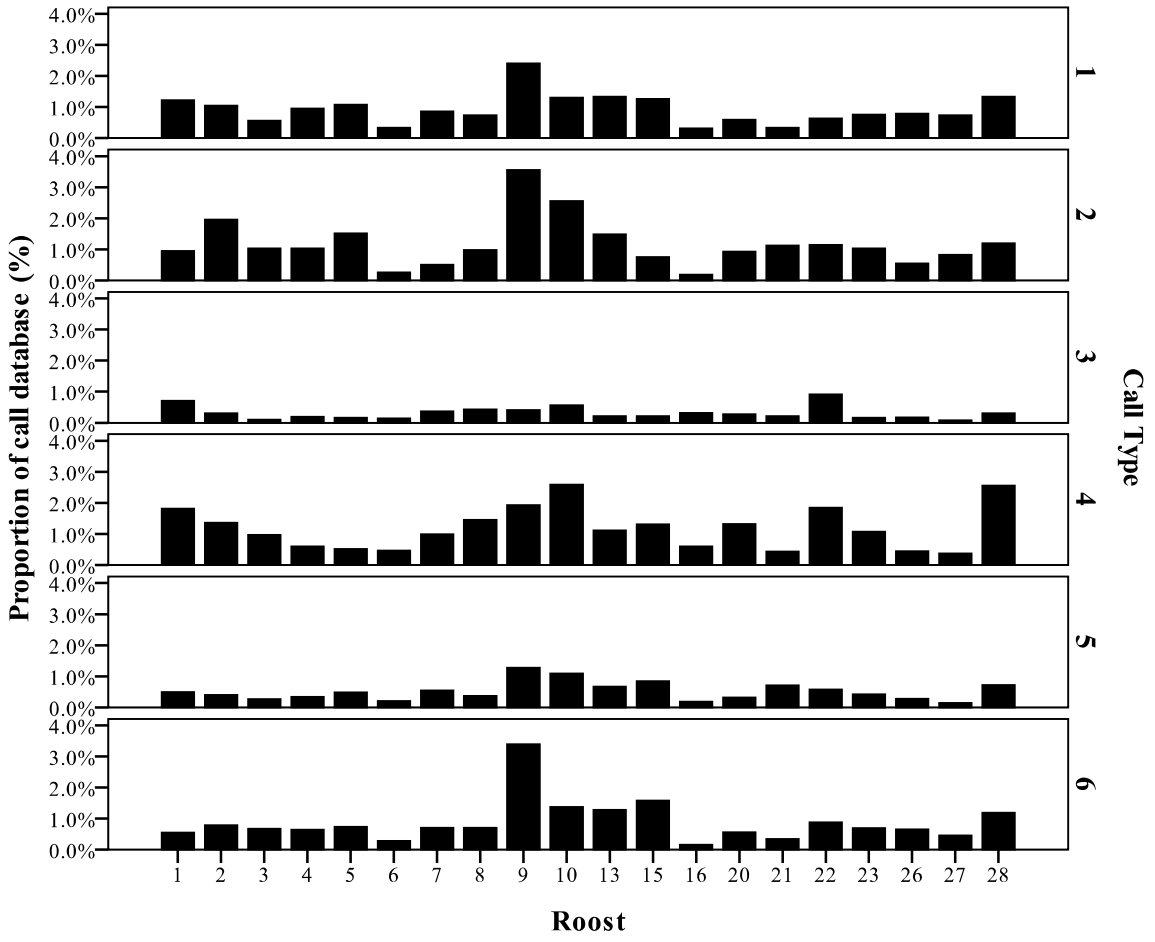


Figure 3.14: The proportion of Type A calls that each of the six call types accounted for at each of the 20 maternity roost sites across Sussex.

4.1 - Introduction

P. auritus produce a variety of social calls. These are produced frequently at maternity roost sites, particularly around the times that coincide with bats leaving or returning to the roost (as demonstrated in Chapter 3). Social calls are also given by bats away from the roosts but they are given much less frequently, but the functions of these calls are largely unknown.

One method of studying the function of vocal signals is to use playback experiments in which recordings of calls are broadcast, and responses of one or more animals to those calls are monitored. Playback techniques have been used to study call function in various animals, in particular birds (for review see Douglas and Mennill 2010) but, also in primates (Norcross et al., 1994; Semple, 1998; Smith et al., 2009), and other mammals, for example lions (McComb et al., 1993) and North American red squirrels *Tamiasciurus hudsonicus* (Digweed and Rendall, 2009). Playback has also been used to investigate the behavioural responses of bats to a variety of calls, including both echolocation (Barclay, 1982; Gillam, 2007; Jonker et al., 2010; Voigt-Heucke et al., 2010) and social calls (Balcombe and McCracken, 1992; Barlow and Jones, 1997; Russ et al., 1998; Wilkinson and Boughman, 1998; Knornschild and Von Helversen, 2008).

Conducting playback experiments at colony roost sites might be problematic because of the social complexity of the situation. There would be high levels of background noise from the bats' own calls, and it is likely that multiple bats would respond to the same stimulus, both of which might confound interpretation of the results. There is also the risk that playback

experiments would disturb the bats and lead them to abandon the roost. Playback experiments at foraging sites are less likely to face these problems.

Recording how *P. auritus* respond to playback of social calls at woodland sites may help us to understand the function of these calls away from roost sites. However, as it is often difficult to obtain high quality recordings of *P. auritus* away from roost sites (as social calls are produced so rarely), simulations of *P. auritus* social calls were used as an alternative method to produce high quality calls with little interference. The Autobat is a portable ultrasound synthesiser that can be used to simulate vocalisations of bats. It was originally developed to be used as an acoustic lure to attract bats for survey purposes, particularly in woodland habitats where bats are otherwise difficult to catch. Its effectiveness has been demonstrated experimentally (Hill & Greenaway, 2005), and it can be used to attract various species (Hill & Greenaway, 2009).

The experiments described in this chapter aim to investigate why bats respond to the Autobat stimulus by (i) comparing the bats' responses to simulated calls of their own species, another species and mechanical noises in a comparable bandwidth, (ii) assessing whether responses vary seasonally and between sexes, and (iii) analysing the nature of behavioural responses the bats show to a range of stimuli modelled on various social calls of their own species.

4.2 - Pilot Study: Filming behavioural responses to simulated social calls in the field.

4.2.1 - Introduction

The main aim of the pilot study was to determine the feasibility of recording behavioural responses of *P. auritus* to simulated social calls in the field, and whether it would be possible to obtain a usable estimate of how close a responding bat approached the source of the stimulus (Autobat speakers). The pilot study also aimed to identify a suitable latency interval for deciding when one response ended and another began.

4.2.2 - Methods

4.2.2.1 - Pilot study filming and data collection procedure

For the pilot study 18 recording locations were chosen at five sites, across two counties, in East and West Sussex. Recording locations at the same site were situated at least 200 metres apart in order to minimise the likelihood of the same bat being recorded at different locations. The Autobat speakers were suspended from a horizontal tree branch, at a height of approximately three metres, using metal hooks. The output from the Autobat was broadcast as ultrasound using two Senscomp/ Polaroid Series 600 Environmental Grade transducers. The output from these transducers is highly directional, with a beam of about 15^{O} at 50kHz, -6dB (Senscomp, 2004). One way of increasing the area over which the output is broadcast is to mount transducers on a pole and rotate the pole by hand (Hill & Greenaway, 2005), but it is not practical to do this for long periods, or when the behavioural responses of the bat are to

be recorded. To overcome this problem an automated system was built by Frank Greenaway that incorporates a rotating deflector to distribute the ultrasonic signal, as described below.

The circuitry is housed in a plastic box (12.5x8x3.5 cm). The transducers are mounted vertically, above the box, 8 cm apart and facing one another. Midway between them is a square vane (4.5x4.5cm) made of 0.5mm polished aluminium, which is attached to the spindle of a 12v electric motor. Power supply to the motor is controlled by a pulse-pause timer (Velleman Mk111) that allows both the pulse length and the interval between pulses of power to the motor to be adjusted. When the vane is perpendicular to the transducers it had no effect on the direction of the ultrasonic output. When it is parallel it reflects sound directly back to them. However, in all other parts of its rotation the vane serves to deflect the ultrasound of each transducer in a direction determined by the resting position. The resting position of the vane changes after each spin and is not controlled in anyway.

For the current study the interval between the start of each pulse was set at 2.5 seconds, and the pulse length was adjusted so that the vane rotated rapidly for about 0.25 seconds, during which time it completed three to five rotations before coming to rest. A test trial of 240 successive spins found that the number of times the vane came to rest in each of six 30^{o} sectors did not differ from what would be expected by chance (χ^2=7.15, df=5, p=0.210). Therefore, the system should produce a fairly even distribution of the ultrasound output in the area surrounding the speaker box.

Two Sony Handycam (Model DCR – DVD91E) digital video camera recorders were set up to record responses to the Autobat. One camera was placed directly beneath the speakers, on a tripod approximately 0.5 metres from the ground recording the bat's location in the horizontal plane, and the second camera was positioned approximately two metres away from the speakers, on a tripod approximately 1.5 metres from the ground, recording the bat's location in the vertical plane. Two infra-red lights (Model Envin micro 75L) were used to illuminate the area around the speakers. A synthesised social call, stimulus A, (Figure 4.1) comprised of the same sweep frequencies repeated five times, (but the third and fourth sweeps are attenuated) was then produced using the Autobat and the bats that responded to the stimulus were captured on video. Their vocal responses were recorded using a Petterson D240x bat detector and a Sony portable MD recorder (Model MZ-RH710). Using headphones with the D240x allows heterodyne output to be monitored in one ear, while the time expansion output can be heard in the other. Whenever a suspected social call was heard in heterodyne, the time expansion trigger was pressed, and 1.75 seconds of sound was played with a time expansion factor of ten. If the output included one or more social calls of *P. auritus*, it was then recorded onto the minidisc player. The Autobat broadcasted call A for a period of five minutes followed by an interval of five minutes of silence. A total of 33 two hour filming sessions were (each split between two locations at each site) took place from May 2006 to September 2006. Filming had to be curtailed on three occasions to a one hour session due to rainfall.

4.2.2.2 - Pilot study data analysis

The data from each filming session was downloaded to a personal computer (Dell Inspiron 2200) and analysed using Sony Picture Package Version 1.8 for DVD Handycam. An

inherent difficulty when recording and measuring behaviour is how to determine when one behaviour responses ends and another begins. The response, in this instance, refers to a bat being recorded on video in the experimental area regardless of whether a stimulus was being broadcast. For example, if a bat responds to a stimulus for a ten second period but flies away from the stimulus twice during that period, should that be recorded as a single ten second behavioural response or as three shorter behavioural responses? In principle, the shorter the interval the more accurate a time-sample recording will be. However, the shorter the interval the less likely it is to record reliably several categories of behaviour if they occur in unison, especially if the activity is complicated or occurs rapidly (Martin and Bateson, 2007). The frequency the responses were plotted against the interval. If behaviours are clumped, with many behaviours occurring within short intervals and few occurring at longer intervals, then the point at which the slope of the line changes most rapidly corresponds to the break point (Slater and Lester, 1982). Slater & Lester (1982) recommended that a slightly longer interval may be more accurate than the one corresponding exactly to the break point.

4.2.2.3 - Pilot Study Behavioural analysis

Once the response interval time was ascertained, each response was edited into a separate file and the behaviour observed and noted. This included the type of flight, a general impression of the size and shape of the responding bat. Ultrasound recordings were analysed using Batsound (v.3.3.1 Pettersson Elektronik AB, Uppsala) and the recordings were time matched with each response (where possible). A preliminary categorisation of behaviours was made, the results of which are not reported separately but which were used to inform the full categorisation of behaviour (detailed in Section 4.4.4.3).

4.2.2.4 – Estimating the proximity of the bat to the speakers

In order to test the feasibility of estimating the proximity of a responding bat to the speakers of the Autobat stimulus, using the speaker box as a gauge, a trial was conducted using a solid object (a suspended tennis ball) placed from 5cm to 100cm at intervals of 5cm from the centre of the speaker box (dimensions: 12.5cm width, 11cm length and 4cm depth) vertically, and then this was repeated horizontally. The resulting 40 positions of the ball were filmed in daylight and edited into separate files using Sony Picture Package Version 1.8 for DVD Handycam with a note made of the actual distances for each file. The 40 files were randomised and the distance of the ball from the speaker box was estimated for each of the 40 files. The estimated and actual distances for each file were subsequently compared in order to assess the error rate using a test-retest reliability statistic.

4.2.3 - Results

4.2.3.1 - Pilot Study: filming behavioural responses

A total of 33 video recording sessions were carried out across 18 sites from May 2006 to September 2006, which resulted in 66 filming hours. Bats frequently flew out of view for a brief period and then back into view again, so it was necessary to set a criterion by which to decide whether such occurrences would be counted as one response or two. The time interval was measured between each pair of successive records to the nearest second. Figure 4.2 shows the frequency distribution of interval lengths up to a maximum of 20 seconds, which

included 95% of all intervals between responses recorded in 2006. The break point, indicated by the red line, is approximately seven seconds but, according to Slater and Lester (1982, the best estimate is a slightly longer interval than the one corresponding to the break point, so a conservative estimate of nine seconds, indicated by the blue line, was applied as the criterion for minimum time interval between responses. This resulted in a categorisation of 208 separate responses that were used for analysis. There is the possibility that two short responses may have been categorised as one longer response. For example, a short response where a bat appeared on film for three seconds, an interval of five seconds with no bat on film, followed by a subsequent short response of three seconds where the bat was on film. This is unlikely to have occurred very frequently, as 83% of the times a bat flew into the view of the camera, the time intervals between successive responses were less than five seconds. Field notes also indicate that the responding individual more often than not flew in the vicinity of the Autobat during the response, but flew around trees, which may have resulted in the responding individual being obscured from the view of the camera lenses. Responding individuals may also have been missed from individuals approaching from behind the horizontal camera.

4.2.3.2 - Approximating the distance the bat approached the stimulus

The intra-observer reliability test coefficient indicated that the estimated distance of an object (the tennis ball) from the speakers, using the dimensions of the speaker box as a reference guide, gave a fairly reliable estimate of distance (Pearson Correlation $r = 0.992$, $n = 40$, $p < 0.01$). The estimates of distances were correct to 10cm all cases and, therefore, it was concluded that it was possible to estimate an approximate distance between the responding bat and the stimulus. Figure 4.3 shows a still photo of a bat responding to the stimulus within

12.5 cm of the speaker box in the vertical plane. Figure 4.4 shows a still photo of the same bat responding to the stimulus within 25 cm of the speaker box in the horizontal plane. Taken together, it was possible to estimate the approximate distance that a bat approached the stimulus.

4.2.3.3 - Responses to a P. auritus *simulated social call*

There were significantly more bat responses filmed, per hour, when the Autobat was broadcasting social calls than when the Autobat was silent (Wilcoxon n = 66, z = - 4.878, p=0.0001), shown in Figure 4.5. There was also a significant difference between season in the mean response rate per hour when the Autobat was broadcasting a stimulus social call (Kruskal-Wallis ANOVA H = 14.882, n = 5, p < .005). Post hoc pair wise comparisons were made (using Mann Whitney U with a Bonferroni correction to reduce Type 1 error rate (.05/10) resulting in a critical value for significance of Z = 2.81, p = 0.005) to investigate where the differences in response rate per hour were significant and the results are detailed in Table 4.1. There were significantly more responses per hour in both July and August compared to September and significantly fewer responses per hour observed in May compared to June, July and August. In contrast, there was no significant seasonal difference in the response rate per hour when the Autobat was silent in the two minute intervals between the three minutes of stimulus broadcast (Kruskal-Wallis ANOVA H = 2.372, n = 5, p = 0.668.). Furthermore, responses recorded in this period may be as a result of latency in response to the stimulus (i.e. some of the bats filmed in these intervals may actually have been attracted by the Autobat, but were reluctant to approach too close while it was broadcasting). Of the 29 responses that occurred when the Autobat was silent, 15 occurred

within the first minute of silence, three in the second, four in both the third and fourth minute and three in the fifth minute. Although there were more responses that occurred in the first minute of silence after stimulus A was terminated, this difference was not significant (Kruskal-Wallis ANOVA H = 3.535, df = 4, p = 0.473).

P. auritus were the most frequently recorded responding species, as shown in Figure 4.6, with a total of 143 responses (response rate per hour 4.81, SD = 3.22, n = 143), recorded from May to September 2006. Ultrasound recordings, both echolocation and social, were obtained for 73 of the *P. auritus* responding to the stimulus. The remaining 70 responses were identified as *P. auritus* on the basis of morphology (both ear and wing shape) and flight pattern (slow and fluttering). There was the likelihood that *P. austriacus*, if responding, could have been misidentified as *P. auritus*. However, this considered unlikely as *P. austriacus* had been captured at only one of the woodlands, Ebernoe Common, and this was a capture of a single individual. Furthermore, radio-tracking of the bats from the colony that this individual came from showed that this species had a distinct preference for more open habitats, such as meadow and pasture (Frank Greenaway & Daniel Whitby, pers. comm.). *P. austriacus* preference for more open habitats has also been reported by radio-tracking studies on the continent (Fluckiger and Beck, 1995; Sevcik, 2003). It should be noted, however, that the use of both mixed and deciduous woodland has also been reported (Barataud, 1990).

A total of 42 responses (1.27 per hour, SD = 1.15, n = 42) could not be identified to species level. This was because no ultrasound recording was obtained in these cases. 15 responses (0.42 per hour, SD = .66, n = 15) were identified as those of *Myotis* species. It was not possible to identify most species of this genus due to the overlapping parameters of their

echolocation calls. It was possible, however, to identify Natterer's bat *Myotis nattereri* as this species has lower end frequencies (i.e. bellow 20kHz) in their echolocations calls than other species in this genus (Russ, 1999; Jones and Waters, 2000; Jones and Holderied, 2007) Eight of the fifteen responses identified as those of the *Myotis* genus were therefore confirmed to be *M. nattererii*. Seven responses (0.21 per hour, SD = .54, n = 7) were identified as soprano pipistrelle *Pipistrellus pygmaeus* and 1 response (.03 per hour, SD = .17, n = 1) was identified as common pipistrelle *Pipistrellus pipistrellus.*

4.2.3.4 - Vocal responses to the stimulus

During the recording of behavioural responses on video, 73 recordings were made of *P. auritus* vocalisation sequences. Of these 46 were exclusively echolocation sequences, 22 included both echolocation and social calls, and five recordings were exclusively social calls. Therefore, a total of 27 out of the 73 *P. auritus* responses recorded (37.0%) included a social call that was recorded during the filming period. Figure 4.7 shows a sonogram of a *P. auritus* responding vocally to the Autobat.

4.2.4 - Discussion

The pilot study demonstrated that it was feasible to record behavioural responses of *P. auritus* to simulated social calls in the field using infra-red video and that it was possible to ascertain the approximate minimum distance between the responding bat and the stimulus.

The pilot study also provided the basis for setting nine seconds as the minimum interval required to classify successive observations as separate responses.

In this pilot study there was evidence of other species being attracted to the stimulus, in particular species from the genus *Myotis* and, specifically, *Myotis nattereri*, which suggested that, in addition to intraspecific responses, there may also be responses that represent interspecific responses. *M. nattereri* is a species frequently associated with woodland habitats (Parsons and Jones, 2003; Smith and Racey, 2008) and, like *P. auritus*, gleans to capture prey items (Siemers, 2001; Swift and Racey, 2002). However, in contrast to *P. auritus*, *M. nattereri* rarely uses prey-generated sounds to detect prey and is able to find prey by echolocation very close to vegetation (Siemers and Swift, 2006).

Approximately 20% of the responses could not be identified to species level in most cases because there was no time expansion recording made at the time of the bat responding. Less than 56% of the filmed responses of *P. auritus* had an ultrasound recording accompanying the responses and almost 37% of these recordings included a *P. auritus* social call. The use of an alternative recording system, such as a continuous automated ultrasound recorder, may achieve improved identification of responding bats and maximise the chances of recording vocal responses to the stimulus.

4.3 – Investigating the capture rates of *Plecotus auritus* to the Autobat using different stimuli

4.3.1 - Introduction

The pilot study showed that *P. auritus* responded to simulated social calls but there was evidence that other species also responded. Studies by Hill & Greenaway (2005, 2008) found that *P. auritus* was the species most frequently captured when simulated *Motis bechsteinii* social calls were used as an acoustic lure. These observations raise the question of whether bats approach the stimulus because of its resemblance to a social call of their own species, or if the response represents investigation of a novel source of ultrasound in the environment. If the similarity to a *P. auritus* social call is important, then the response rates to these calls should be higher than to social calls of other species, or to other stimuli of similar frequency and intensity.

Like many other species, *P. auritus* frequently responds to simulated social calls by rapidly approaching the speakers, which is why the Autobat is effective as an acoustic lure (Hill and Greenaway, 2005; Hill and Greenaway, 2008; Schöner et al., 2010). This means that capture rates may be used as a crude measure of responsiveness to different stimuli. An experiment was, therefore, conducted in which capture rates to simulations of *P. auritus* social calls were compared with those using other stimuli.

4.3.2 - Methods

Trapping took place at ten sites in six woodlands in West Sussex and Surrey. Each trapping site was used only once. Where multiple trapping sessions took place in the same woodland they were separated from one another by at least 200 metres. At each trapping site two harp traps were set in the interior of broadleaved woodland at least 150 metres apart. Harp traps were used in preference to mist nets as the traps could be left unattended for brief periods, which enabled the experimenter to change the calls and check the traps. Furthermore, harp traps are considered to be especially effective in catching small bats that weigh less than 30g (Kunz and Kurta, 1988; Francis, 1989).

Two Autobats were programmed with three different simulated stimulus calls and one Autobat was placed adjacent to each harp trap. The ultrasonic transducers (Polaroid Series 600 Environmental Grade) which broadcast the simulated ultrasound calls, were mounted on a pole so that they were parallel to the bank of strings on the trap. The experiment began at least one hour, but less than two hours, after civil twilight. The three stimulus calls, shown in sonogram Figure 4.8, were played in an irregular sequence of five sweeps (each sweep between 11 and 12 milliseconds in duration) following the same temporal pattern for each call, with the same intersequence interval. Each call was played for 30 minutes in rotation and the bats captured in response to each call type were recorded. A Fisher exact probability test (2 x 3 contingency table) was used to compare the capture rates of *P. auritus* to each of the 3 call types, using the statistical software SPSS®.

4.3.3 - Results

Plecotus auritus was captured in the harp traps when responding to simulated social calls of their own species on eight nights out of ten, compared with only two nights when capture coincided with simulated *M. bechsteinii* social calls, and one night when a capture coincided with the non-bat ultrasonic noises, as shown in Figure 4.9. Simulated *P. auritus* social calls were significantly more effective at capturing *P. auritus* bats than either simulated *M. bechsteinii* calls or ultrasonic non-call noises (Fisher Exact Probability 2 x 3 p = 0.0009).

4.3.4 - Discussion

P. auritus responded on significantly more nights to the simulated *P. auritus* social call than to the simulated *M. bechsteinii* call or to the non-call stimulus. This supports the hypothesis that the response of *P. auritus* to the stimulus is a social one elicited by the similarity of the stimulus to a conspecific's social calls. A study by Russ, Jones and Racey (2005) which played back distress calls of soprano pipistrelles *Pipistrellus pygmaeus* also found that bats responded significantly more frequently (based on the number of echolocation recordings) to the base calls as opposed to control calls (random noise) or calls that had been experimentally modified to differ in frequency, interval and repetition rate (Russ et al., 2005).

If the responses to the stimulus did represent an investigation of novel sound, then no significant difference would be expected in the frequency of responses occurring to each of the three stimuli. If it was a generalised response in which the bat investigates something that sounds like a bat social call, then similar levels of response to both of the bat call stimuli

would be expected. The fact that simulations of the own species calls were most effective suggests that the similarity to a conspecific's call was a key factor.

In this study *P. auritus* was captured in response to the *M. bechsteinii* call on one night only. However, an extensive two year study to survey for *M. bechsteinii* across southern England (using simulations of *M. bechsteinii* social calls) found that *P. auritus* were captured frequently in response to this call type (in 62% of the woodlands surveyed in 2005 and in 74% of the woodlands surveyed in 2006), which suggests that response to simulations of other species calls does occur frequently. This experiment was limited as only three stimuli calls were tested and it may be that other stimuli that do not resemble *P. auritus* social calls could be equally as effective. Furthermore, only the individuals that were captured in the harp trap were recorded and, therefore, responses that did not involve a close rapid approach would not have been recorded.

4.4 – Assessing the sex differences in responses to the Autobat

4.4.1 - Introduction

In studies of vocal communication that use playback experiments, knowledge of the sex of the responding individual can help to elucidate call function (Semple, 1998; Smith et al., 2009; Douglas and Mennill, 2010). In animals that are diurnally active, sex can often be readily determined from visible physical differences. However, because bats are small, fast-flying and active at night, it is generally impossible to determine the sex of an individual without inspecting it in the hand. Therefore, an inherent difficulty with studying infrared video behavioural responses of bats to simulated social calls in the field is that, not only is it

difficult to accurately identify the species, it is impossible to determine the sex of the responding individual using infra red video alone.

The pilot study (Section 4.2) showed that *P. auritus* frequently responded to simulated sequences of a *P. auritus* social call, termed 'stimulus A'. However, the sex of the individuals responding was unknown. The two main aims of this study were to (i) compare the sex composition of bats captured by the Autobat throughout the season and to (ii) analyse whether there are behavioural differences between male and female responses to the stimulus by differential marking of captured bats.

4.4.2 – Experiment 1: Sex differences in capture rate when using the Autobat

4.4.2.1 – Experiment 1: Methods

A capture study was conducted at Plashett Wood, Clayhill Wood and Hemsley's Rough in East Sussex. The capturing of the bats was carried out over an entire season (May to September 2007) to provide data on whether there are seasonal differences in relative capture rates of males and females. Eleven capture locations (the positions of which are shown in in Appendix A.4.1) were chosen in the three woodlands. The 11 capture locations were situated in the areas where the most *P. auritus* responses had been recorded in 2006 (and in trial filming sessions in April and May 2007) from a total of 17 locations across the three woodlands. The bats were captured at the woodland sites over 23 nights between May and September 2007 using a combination of harp traps and mist nets, to which they were attracted using the Autobat stimulus A. Each site was trapped twice, but site nine was netted three

times as a high number of bats were captured at this location. Trapping was suspended for two weeks around the birth period, to avoid capture of bats in late pregnancy or early lactation.

4.4.2.2 – Experiment 1: Results

A total of 32 female and 20 male *P. auritus*, (detailed in Appendix A.4.2), were captured across the 11 different locations in the three woodlands in 2007. There were more female bats captured (a total of 32 over 23 nights of netting and trapping) than males (a total of 20 over 23 nights of netting and trapping) but this difference was not significant (Mann Whitney U test $Z = -1.772$, $n = 58$, $p = 0.076$). However, when the mean monthly capture rate per session for both males and females was plotted, as detailed in Figure 4.10, it was clear that there was a seasonal difference in the captures of males and females. A two-way ANOVA was carried out to test the effect of sex and month on the number of captures. This showed that there was no significant difference in the capture rates of bats for each month and no significant difference between the capture rates of males and females (as indicated above), but there was a significant effect of an interaction between month and sex, as shown in Table 4.2.

4.4.3 – Experiment 2: Differential marking of male and female bats

4.4.3.1 – Experiment 2: Methods

Each of the individuals captured (as detailed in Appendix A.4.2) was marked with a reflective metallic band. Marking each sex with a reflective band on opposite wings, allows for differentiation between males and females responding to the Autobat on infra-red film,

should they subsequently respond to the stimulus and be captured on film. In addition to the bats captured at the 11 woodland sites, seven female and four male bats were captured from a maternity roost in a house at Plashett Park Farm. This roost location was found during a pilot study in Plashett Wood in 2006 in which bats were captured and radio-tracked back to their roost (see Chapter 5). The bats were captured in a pole trap as they exited the roost. This roost was only trapped once, using this method, as frequent trapping at roost sites could potentially cause roost abandonment (Mitchell-Jones and Mcleish, 2004).

Aluminium-alloy 3mm bat rings (Mammal Society London) were covered in cyanoacrylate adhesive and a piece of reflective tape (Sakuma Retro Reflective Tape) was attached to each of the metal bands. One disadvantage of reflective tape to bands is that it is often chewed off by the bats, making regular replacement necessary (Kunz, 1996). Therefore, at least five coats of non-toxic water based varnish (Acquarella) was applied to seal the tape on to the band and reduce the likelihood of the bats removing it.

Female bats had a reflective wing band fitted around their right forearm and male bats had a reflective wing band fitted around their left forearm. After each night of capture at a site where bats were marked, a night of filming responses to the Autobat (over a period of two hours per night) followed within a minimum of five days after the capture took place. The filming of responses was carried out using the methodologies (detailed in Section 4.2.2.1) and the number of responses from bats with reflective banded wing bands was noted.

4.4.3.2 – Experiment 2: Results

A total of 44 hours of filming resulted in 39 responses of individuals with reflective bands which included 34 female responses, five male responses and 254 responses of *P. auritus*

with no reflective band. This resulted in a mean response of 0.866/hr of banded bats, which was lower than the 5.77 *P. auritus* responses per hour recorded for non-banded bats. Of the 34 responses by females, 25 were by seven radio-tagged animals. This was because these radio-tagged individuals were targeted by broadcasting the Autobat within their home range (see Chapters 5 and 6 for detailed analysis of radio-tracking study and how radio-tagged females responded to the stimulus). The remaining nine responses were recorded at five different sites (one response at Clayhill Wood, six responses at three sites in Plashett Wood and two responses at Hemsley's Rough). The male responses were recorded at two locations (four responses recorded at Clayhill Wood (all on the same night) and one response at Hemsley's Rough site 15). The low response rate recorded for males with reflective bands means that statistical comparison with responses of marked females was not possible.

4.4.4 - Discussion

There was no overall significant difference in the capture rate between the number of male and female bats. There was, also, no overall significant difference between months in the capture rates for both sexes. However, female bats were caught significantly more frequently earlier in the season and male bats were caught significantly more frequently later in the season. This seasonal difference between male and female bats has been observed in capture studies of bats in forests, without using lures. For example, an eight year study of mist netting study in the Ouachita Mountains in Arkansas found that the sex ratios of eastern red bats *Lasiurus borealis* captured were predominately male in late summer but were dominated by females in mid-summer (Perry et al., 2010). The authors suggested that the greater proportion of females found mid-summer may have reflected an increase in activity of females due to the demands of reproduction.

25 of the 34 responses by females (over 73%) were from seven bats that had a radio-tag fitted as well as a reflective band fitted. The remaining nine responses were recorded at five separate locations, which, based on radio-tracking data, are more than likely to have been responses recorded from at least five different females. This could mean that at least 12 of the 39 females (approximately 30%) were subsequently recorded responding to the stimuli on infra-red video after they had been captured and fitted with reflective bands. However, by comparison, a total of five male responses were recorded at only two sites (and four of the responses were recorded on one night, which could easily have been multiple responses of the same bat). This could mean that possibly only two of the 24 males (approximately 8%) were subsequently recorded responding to the stimuli on infra-red video after they had been captured and fitted with reflective bands, suggesting that, in comparison to females, males may be less likely to respond to the stimulus, perhaps as a result of been captured previously or, potentially because they may have larger foraging ranges.

Reflective bands, to enhance the visibility of flying bats at a distance or while in the roost, have been used with varying degrees of success by bat researchers. Some studies have found that the use of reflective bands was successful in identifying foraging bats at a distance (Bradbury and Vehrencamp, 1976; Humphrey et al., 1977; Racey and Swift, 1985; Rydell, 1989). However, these were for bats that tend to use more open habitats to forage than *P. auritus*. A study that compared the foraging habitats of *P. auritus* and *M. daubentonii* occupying the same roost, found that the bats marked with reflective bands could only be seen at short distances (Swift and Racey, 1983).

The use of metallic bands to mark bats needs careful consideration as there are potential welfare implications. Metallic bands can affect mobility and even cause direct injury if

inappropriately applied. However, Dietz et al., (2006) compared published and unpublished injury rates of 28 bats species and found that the injury rate for European vespertilionid bats, including *P. auritus*, marked with metal bands since the 1990's was generally below 5%. Only *Pipistrellus nathusii* showed a higher rate of injury at 6.3%.

The differences in behavioural responses of male and female bats may be better addressed using alternative methodologies (such as, for example, radio-tracking) given the low-recapture rate of bats with reflective bands responding to the Autobat on infra-red video and, also, the potential harm to the individuals (although this was considered to be low). It may have been possible to have captured a high proportion of the bats at known maternity roost sites surrounding Plashett Wood but, based on colony counts (see Chapter 3), this would have been in the region of 200 individuals and it was considered that this may have been too invasive in terms of the local population. It may be possible, however, to apply the technique (using the Autobat) in smaller woods, as there would be a greater probability of ringing a higher proportion of the population, yielding more informative results.

4.5: Behavioural responses of *P. auritus* to a variety of simulated social calls.

4.5.1 - Introduction

The pilot study (Section 4.2) used a single stimulus 'stimulus A' and study two (Section 4.3) showed that simulations of an individual call from the 'stimulus A' sequence was more effective at catching *P. auritus* than a simulation of an individual call from a *M. bechsteinii*

call sequence or a simulation of an ultrasonic noise. However, *P.auritus* used a wide variety of social calls, as demonstrated by the variety of call types recorded at the roost sites (as detailed in Chapters 2 and 3). Various calls have also been recorded in the field away from known roosts sites. Six of these calls had been found to be effective as stimuli for the Autobat acoustic lure (unpublished observations by Hill, Greenaway and Murphy). The main aim of this experiment was to assess whether differences were apparent in the nature of the bats' responses to the different types of synthesised call, whether these responses varied seasonally and whether changing the volume of the calls influenced the bats' responsiveness.

4.5.2 – Methods: Filming behavioural responses to a variety of stimuli

4.5.2.1 – Experiment set up and data collection procedure.

A total of 38 filming locations were selected in 11 woods in East and West Sussex and Surrey. Filming locations in the same wood were situated at least 200 metres apart in order to minimise the likelihood of filming the same bat at different locations. The experimental set up followed the procedure previously described for the pilot study (Section 4.2.2.1) with the following modifications: (i) the second camera, mounted horizontally, was placed three metres away from the stimulus in order to increase the filming area and capture more prolonged, complex behaviours; (ii) an automated broadband ultrasound recorder was used to continuously record all ultrasound in the vicinity of the Autobat speakers to improve the identification of species of bats responding; and, (iii) each call type was played for a period of three minutes with a two minute interval of silence between each call. This was to allow a sufficient latency period between calls (to correctly assign the response to the appropriate call), to ensure equipment was still recording and change film discs and batteries when

required and, also, to allow time for a sufficient variety of calls to be used at each filming session. A total of 172 hours of filming was carried out across the 38 locations from May 2007 to September 2007 and May 2008 to September 2008.

4.5.2.2 Simulated social calls

Six social calls were used as models for the stimulus simulations (calls A, C and D were recorded by David Hill & B, E, and F were recorded by Stephanie Murphy from free flying bats away from roost sites in 2006). For each stimulus the intervals between individual sweeps in a sequence were based on those of actual recordings, but the sequences themselves were repeated at much higher rates than have been recorded in the field. Ultimately, the patterns and rates of the sequence repetition were ones that had proved effective in attracting bats for capture. Consequently, it could be regarded as a super-stimulus, rather than an attempt to simulate the natural communication between *P. auritus*. Sonograms of the six stimuli used are shown in Figure 4.1 (stimulus A previously shown in Section 4.2) and Figures 4.11 to 4.15 (stimuli B – F). The calls differed in intensity and temporal patterning as well as structure. The intensity of each call was measured at the frequency of maximum energy for that call. The method by which intensity was measured and the equipment was calibrated is described in Appendix A.4.3. The acoustic parameters, temporal patterning and intensity measurements for each of the stimuli are detailed in Table 4.3.

Each stimulus was played for three minutes followed by an interval of two minutes of silence, and then another stimulus was played. All stimulus types were played both amplified and non-amplified (so that stimulus A with amplification was AL (loud), and stimulus A without

amplification was AQ (quiet)). The 12 stimuli were played in a randomised order (using the random number generator function in excel) changing the order that the stimuli were played each night. After a period of one hour, each stimuli having been played once amplified and once unamplified, the filming was terminated, the location moved, and the process repeated.

4.5.2.3 - Behavioural categorisation

The data from each filming session was downloaded to a Personal Computer (Dell Inspiron 2200), edited using Sony Picture Package Version 1.8 for DVD Handycam, and analysed in slow motion using VLC Media Player Version 1.1.4. For each night's filming the following details were recorded for each occurrence of a bat recorded on video: (i) the time of occurrence; (ii) the length of time for which the bat was visible; (iii) the estimated distance of closest approach to the speaker box; and (iv), a preliminary description of the behaviour. The edited behavioural sequences were collated and then analysed, repeatedly, with a view to placing each into a behavioural category.

The observed behaviours were separated into discrete categories, ensuring that the definitions of behaviour were clear, comprehensive and unambiguous. When choosing the observational categories that a response should be allocated to, the following guidelines (as recommended by Martin and Bateson 2007) were adhered to: (i) enough categories were available to describe each response in detail; (ii) each category was precisely defined and summarised with as much information as possible about the behaviour; (iii) categories were discrete (two or more categories did not describe the same thing); and (iv) behaviours within categories were uniform. After numerous examinations of the recorded behaviours and trials of

categorisation systems using 'within observer' reliability techniques to check each outcome (Martin and Bateson, 2007), a dichotomous key was constructed, as shown in 4.16. The description for each category was defined using criteria that could be readily understood by people unfamiliar with bats or their behaviour. Behaviours recorded during the experimental period are termed as a "response" below, regardless of whether the stimulus was playing or not.

4.5.2.4 - Assessment of the classification system

In order to assess the suitability of the behavioural categorisation system, an inter-observer reliability test was conducted using five scorers. Training scorers to allocate behavioural observations to specific categories is often difficult, yielding inconsistent results if the descriptions of categories or behaviours are open to interpretation by the scorer. The use of a binary key reduces the 'training' process but the scorers were given a brief training session, working through two filmed responses and using the binary key with the author. The scorers included three people with backgrounds in biological science (one post-doctoral and two PhD candidates) and two people with little or no scientific background. None of the scorers had any previous experience of studying bat behaviour.

The scorers were provided with a score sheet (shown in Appendix A.4.4), a definition sheet (shown in Appendix A.4.5), and the behavioural binary key (as shown in Figure 4.16). Each observer was given the same 50 edited behavioural observations, and these 50 observations were then split into two sets of 25 that were undertaken as separate tasks to reduce the risk of observer fatigue. The 50 observations chosen included eight from each of the categories (as

defined by the author in Figure 4.16) to ensure that there was a uniform distribution of the behavioural observations assigned to each category type (chosen from each category using a randomised number table) and two behavioural observations that could not be assigned to a category by the author. The scorers were asked to categorise them to category level (not subcategory level). The 50 observations were categorised by the author (using the binary key Figure 4.16) and the categorisations of the responses by the scorers were subsequently compared to the author's to ascertain the level of agreement using a binary scoring system, whereby 1 was agreement and 0 was non-agreement. Cochran's Q test was then used to test whether the scorers' classifications of the 50 behavioural responses were in agreement with the author's classifications.

4.5.2.5 - Data Analysis

All the data were entered into SPSS, a software package for statistical analysis. Non-parametric tests were used throughout because categorical data was included and the responses could not be assumed to be normally distributed.

4.5.3 – Results: Filming behavioural responses to a variety of stimuli

4.5.3.1 - Number of bat responses

A total of 87 video recording sessions were carried out across 38 sites during May to September 2007 and during May to September 2008, which resulted in 172 hours and 681 bat responses that were used for analysis. Figure 4.17 shows the mean response rate / hour, for

each month, for when the Autobat was broadcasting the six simulated *P. auritus* social calls and for when the Autobat was silent. There were significantly more bat responses recorded, per hour, when the Autobat was broadcasting social calls compared to the number of responses recorded when the Autobat was silent, and this was significant for each month of recording for both years (May to September) (Kruskal-Wallis ANOVA, $H = 6.818$, $df = 10$, $p = 0.009$.).

It was possible to identify the species or genus of the bat for 627 of the 681 responses recorded. *P. auritus* were the most frequently recorded species with a total of 381 responses recorded on film, followed by 105 responses of species from the genus *Myotis* (*M. bechsteinii, M. daubentonii, M. brandtii, M. mystacinus, M. alcathoe, M. nattereri*), and 86 confirmed responses of *M. nattereri.* There were also 30 *P. pygmaeus*, 19 *P. pipistrellus* and six *N. noctula*. The remaining 54 responses could not be identified to species level (the majority of these were bats seen at a distance on film but there was no corresponding ultrasound recording to assist in identification).

4.5.3.2 - Species response rates per hour

There was a significant difference in the response rate per hour between species when the Autobat was broadcasting the six simulated social calls (Kruskal-Wallis ANOVA $H = 245.640$, $n = 6$, $p < 0.001$), as shown in Figure 4.18. Post-hoc analysis (using Mann-Whitney U with a Bonferroni correction to reduce Type 1 error rate (.05/7) resulting in a critical value for significance of 0.007) was carried out to determine whether the differences were

significant and this revealed that *P. auritus* had a significantly greater response rate per hour compared to any other species, as shown in Table 4.4. Species from the genus *Myotis* and responses from bats that could not be identified to species level had significantly greater response rates per hour than *Pipistrellus pipistrellus*, *Pipistrellus pygmaeus* or *Nyctalus noctula.* In turn, *Pipistrellus pipistrellus* and *Pipistrellus pygmaeus* had significantly greater response rates per hour than *Nyctalus noctula* (test statistics detailed in Table 4.4). Occasionally echolocation calls of *Eptesicus serotinus* and/or *Nyctalus leisleri*, *Barbastella barbastellus* and *Pipistrellus nathusii* were recorded at some of the woodland sites. However, these calls did not coincide with any filmed responses of bats.

4.5.3.3 - Behavioural classification system

Six behavioural categories were defined, with a seventh category (Unclassifiable) added for responses that could not be assigned to any of the six. Each of the behavioural categories was further divided into subcategories according to the details of the behaviour, such as how close the bat approached the stimulus, or whether a behaviour was performed just once or repeated. This produced a total of 23 subcategories. The Cochran's Q test indicated a good level of agreement (96.2%) between the scorers and the author in categorisation of behavioural responses to category level (Cohran's $Q = 113.392$, d.f. 49, $p < 0.0001$), which indicated that the observed filmed behavioural responses could be consistently categorised.

4.5.3.4 - Behavioural responses of all bats responding to the stimuli

Of the 681 responses recorded on video, 654 could be classified into one of the main behavioural categories one to six. The remaining 27 responses (less than 4%) could not be satisfactorily assigned to one of the six main categories and were consequently assigned to category seven 'unclassified'. The responses that could not be categorised were 'unclassifiable' for a number of reasons including: difficulty in judging how near to the stimulus the bat approached; occasions when two or more bats responded together, thereby confounding the results; or occasions when the observed behaviour could not be assigned to a category with complete certainty.

4.5.3.5 - Analysis of P. auritus *behavioural responses*

The responses of *P. auritus* bats included all seven behavioural categories, as shown in Figure 4.19, whereas the behaviour of other species that responded to the stimulus could be categorised into only four categories. Notably, the three behavioural categories that included hovering were unique to *P. auritus*. A chi square test found that there was a significant association between species and response type ($\chi^2 = 185.694$, d.f. 19, $p < 0.001$), but when *P. auritus* was excluded from the analysis no significant difference was found ($\chi^2 = 14.056$, d.f. 19, $p = 0.652$). The 34 responses where it was known that the responding individuals were female (see Section 4.4.3.2) were compared to the remaining 254 responses to examine if there was a significant difference between the two groups of responses. There was no significant difference between the frequencies of behavioural categories for known females and responses for where the sex was unknown (Kruskal-Wallis ANOVA H = 0.115, d.f. = 1,

p = 0.734). A total of 87 (23%) of the filmed responses were of multiple (two or more) *P. auritus* responding simultaneously to the stimulus.

4.5.3.6 - Seasonal variation in P. auritus *response type*

There was evidence of differences in the mean number of responses between behaviour categories, evidence of seasonal differences and, also, evidence of an interaction between season and behavioural response type, as indicated by the results of the Scheirer-Ray-Hare test, shown in Table 4.5. A total of 595 post-hoc pair-wise comparisons were undertaken (using Mann-Whitney U with a Bonferroni correction to reduce Type 1 error rate (.05/35) resulting in a critical value for significance of p = 0.0014) to investigate where the differences were significant. Figure 4.20 details the results of the mean number of responses for each behaviour category in each month.

Excluding 'unclassified' behaviours, there was evidence of seasonal differences in the mean number of responses for each category. In May, category one responses 'approach the stimulus and leave immediately' were observed significantly more frequently than category six responses 'hover and leave'. In June, category four and five responses 'fly near the stimulus moving continuously' and 'fly near stimulus pausing to hover'(behaviours longer in duration), were observed significantly more frequently than responses assigned to categories one and six 'approach stimulus, leave immediately' and 'hover, leave immediately' (behaviours which are shorter in duration). In July, category two and five responses 'fly near stimulus completing at least one 360^{o} rotation' and 'fly near stimulus pausing to hover' were

observed significantly more frequently than responses assigned to categories four and six. There were no significant differences in the mean between categories for observed responses in either August and September.

4.5.3.7 - The effectiveness of stimuli of varying intensity

There were differences in the overall effectiveness of the six simulated calls in eliciting responses, as shown in Figure 4.21, and these differences were significant (Kruskal-Wallis ANOVA $H = 74.032$, d.f. = 6, $p < 0.001$). There were more responses in the silence period compared to stimulus B. It may have been that some of these responses were as a result of a latent response. Of the 33 responses that occurred when the Autobat was silent, 20 of these occurred within the first 30 seconds of silence, which suggests there may have been latency in responses to the previous stimulus broadcast. Table 4.6 details the post hoc analysis (using Mann-Whitney U with a *Bonferroni* correction to reduce Type 1 error rate (.05/7) resulting in a critical value for significance of 0.007) to investigate where the differences between call types were significant. Calls A & D (both high intensity with a high repetition rate) elicited a significantly higher response rate than any of the other call types, but there were no significant differences between call A & D or between the effectiveness of any of the other call types. A comparison of responses by known females (see Section 4.4.3.2) to different call types was compared to the responses of unknown individuals to determine whether the known females were more likely to respond to specific call types. There was no significant difference between groups (Kruskal-Wallis ANOVA $H = 2.997$, d.f. = 1, $p = 0.08$).

4.5.3.8 - Seasonal variation in stimuli effectiveness

There was evidence of an interaction between season and call effectiveness, as shown by the results of the Scheirer-Ray-Hare test detailed in Table 4.7. A total of 595 post-hoc pair-wise comparisons were undertaken (using Mann-Whitney U with a Bonferroni correction to reduce Type 1 error rate (.05/35) resulting in a critical value for significance of 0.0014) to determine whether the differences were significant. Figure 4.22 shows the results of the mean number of responses for each call type in each month. There was evidence of a seasonal difference in call effectiveness whereby in May, calls A & D (high intensity high repetition) were significantly more effective than call C (low intensity high repetition) but not significantly more effective than any other call type. In June, calls A and D were significantly more effective at eliciting behavioural responses than any of the other four calls. In July, call A was significantly more effective than C (but call A was not significantly more effective than any other call type) and D was significantly more effective than calls B (low intensity low repetition) and C. In August, there were no significant differences in the effectiveness of the 6 different calls. In September, call C was significantly more effective in eliciting responses than call B (but call C was not more effective than any other call type). The response rates for silence in August and September were high in comparison to May to July responses rates for silence and higher than to two of the calls in August (although these differences were not significant). This could be as a result of the latency of response for juveniles.

4.5.3.9 – Stimuli amplification

There was no significant difference in the number of responses per hour when responding to amplified and non–amplified calls (Mann-Whitney U = -0.080, n = 348, p = 0.936). The effect of amplification was analysed separately for each of the six social calls, as shown in Table 4.8, but there were no significant differences in the monthly number of responses for each call type between amplified and non-amplified calls. There was a significant association between amplified calls and proximity of overall approach. Close approaches were more frequently observed without the stimuli amplification whereas, in comparison, the distant approaches occurred more frequently when the stimuli were amplified (χ^2 7.54, d.f. 1, p = 0.006).

4.5.3.10 – Association between stimuli and behavioural categories

A highly significant association was found between stimuli type and behavioural category (χ_2 292.357, d.f. 25, p > 0.0001). However, this association test combined all calls and behavioural responses and did not provide any information as to which calls, if any, were more frequently associated with particular behavioural categories. Therefore, post-hoc analysis was carried out using cross tabulation and converting standardised residuals, the differences between observed and expected values, to a 'Z' score which, in turn, were compared to a critical value for alpha. Using a conservative critical value of Z = +/- 3.22 (corresponding to a p value of .0013, using a Bonferroni correction of .05/36 for comparing multiple comparisons), Table 4.10 shows the results of the post hoc analysis of the chi sq test of association. A total of three positive associations and one negative association were

significantly greater than the critical value of Z = 3.22 p = 0.0013. Call A elicited responses assigned to category five, call B elicited responses assigned to category one, and call C elicited responses assigned to category six, all more frequently than expected. By contrast, call D, was less frequently associated with responses assigned to category three than expected.

4.5.3.11 - P. auritus vocal responses

While filming the responses, a total of 481 sequences of vocalisations of *Plecotus auritus* were recorded. These included 251 recordings of echolocation calls only, 269 recordings that were a mixture of echolocation and social call, and 32 recordings that consisted of one or more social calls with no echolocation. A total of 348 *P. auritus* behavioural responses were captured on video whilst the Autobat was broadcasting stimuli. Of the 301 recordings that contained social call elements, 179 of the sequences were recorded whilst the Autobat was broadcasting the stimuli calls, and corresponded to the time when one (or more) *P. auritus* responded on video. There was a significant association between social call production and stimulus broadcast (χ_2 274.32, d.f. 1, $p < 0.001$). However, there was no evidence that any one of the six stimuli (A – F) was more likely to elicit a social vocal response over another call type (χ_2 8.41, d.f. 5, $p = 0.134$).

4.5.4 - Discussion

Behaviour can be viewed as a stream of elements, which, once accurately described, can be counted and timed (Donat, 1991). In order to record patterns of behaviour in a quantitative

manner, they must be broken down into identifiable and mutually exclusive categories that can be accurately assigned. One way of doing this is to use a dichotomous key. The most familiar use of dichotomous keys in biological sciences is in systematics (Metcalf, 1954) but they have also been used in animal behaviour research, for example in studies of hermit crabs *Calcinus tibicen* (Hazlett, 1980) and, also, in studies of mammals such as the common palm civet, *Paradoxurus hermaphrodites* (Krishnakumar et al., 2002), as an identification key to behaviours as it can be faster (and less ambiguous) to use than a list of descriptive behaviours.

The categorisation of a comprehensive description of behaviours for a species is defined as an 'ethogram' (Brown, 1975), which is the result of refining a catalogue of behaviours after many hours of observation and description (Lehner, 1995). The classification of behaviour of *P. auritus* in this study was based on their response to the stimulus, but does not in any way represent a complete repertoire of *P. auritus* behaviours. The classification of the behavioural responses of *P. auritus* to simulated social calls identified a number of different behavioural categories and allocation of behavioural responses to these categories was repeatable, as indicated by the inter-observer reliability tests. The categories differed predominately in observed physical behaviour, proximity of approach, and length of response. One potential disadvantage of categorising behaviours by human eye, however, is that the threshold values are not clearly defined and a small parameter relevant to the animal could be missed by the human (Martin and Bateson, 2007).

Categorisation of behavioural responses to artificial stimuli has been less reported than behavioural observations, but a recent study using video playback investigating the responses of domestic dogs to a robotic model dog with differing tail conditions (short/still, short/wagging, long/still, long/wagging) found that the behavioural responses of the approaching dogs could be categorised and differing behaviours were observed depending on the stimuli used (i.e. long wagging tails were significantly more likely to elicit an approach response compared to sort still tails) (Leaver and Reimchen, 2008).

The behavioural responses of *P. auritus* to the Autobat stimulus were assigned to six main categories within which further sub-categories were defined depending upon how close the responding bat approached the stimulus and which combination of behaviours were subsequently observed in proximity to the stimulus. The behavioural responses of *P. auritus* were frequently characterised by hovering (pause in flight to stay suspended in the air, fluttering in one place) adjacent to the stimulus. Hovering flight is a common habit of *P. auritus* (Norberg, 1976). Hovering was observed in over 53% of the behavioural responses of *P. auritus* whereas it was not observed in the behavioural responses of any other species. Hovering was observed in both short duration responses (category six) for *P. auritus* and, also, in longer, more complex responses (categories three and five). This interspecific difference in response categories is not as a result of hovering being a unique behaviour to *P. auritus*, as hovering has also been reported for *M. nattereri* (Swift and Racey, 2002).

The number of behavioural responses to the stimuli varied seasonally. In May, not only were the responses observed short duration responses (categories one and two), there were also significantly fewer responses to the stimuli. In June responses that were more complex and longer in duration occurred significantly more frequently. Later in the season there were no

significant differences in response categories but, overall, there were fewer *P. auritus* responses recorded to the stimulus in September, compared to August. The variation in the number of responses and types of responses recorded may be as a result of reproduction. For, example the energy demands for *P. auritus* females is greatest in June and July when gestation occurs. Evidence also suggests that offspring, once volant, share feeding areas (Chapter 7).

There are likely to be many factors that influence the nature or strength of an individual's response to stimuli, such as stage in the breeding cycle, presence or absence of offspring or other conspecific, or, potentially, the position of the stimulus within a feeding range of an individual. Female Northern bats *Eptesicus nilssoni*, for example, forage in small feeding areas that are used on consecutive nights. Reproducing females defend feeding territories against other colony members as well as non-members by means of aggressive chases and vocalisations. Intrusions into occupied feeding sites resulted in territorial conflicts in 47% of cases, passive departure by one or more opponents in 43%, or mutual acceptance in 11% of cases. Conflicts occurred regularly throughout the summer, but became less frequent in July when insect abundance increased (Rydell, 1986). In the mating seasons male bats, such as pipistrelle species (Lundberg and Gerell, 1986) and sac-winged bats *Saccopteryx bilineata* (Heckel and von Helversen, 2002), display territorial tendencies, characterised by chasing and vocalising at male intruders.

In this study, the sex of the responding individual was not known for most of the individuals responding to the stimulus, and this prevented conclusions being drawn as to why specific individuals may have responded in such a manner at that time. However, when the subset of the responses for known females was compared to the rest of the responses, there was no

apparent difference in the composition of response type between the groups. If the rest of the responding bats included lots of male responses, then it may be expected to find differences in the composition of responses or, conversely, if only females hovered then it would be expected to find a higher proportion of hovering in the known female subset of responses. No hovering was observed in the male bats marked with reflective bands. However, this sample size was too small to be conclusive.

There were also significant overall differences in the effectiveness of calls of different intensities whereby loud calls with high repetition rates (calls A & D) elicited significantly more responses than quiet calls. This may be explained by that fact that loud, more repetitive calls could potentially be heard by more bats which, in turn, could elicit a greater number of responses. However, if this were true, it would be expected that amplification of these calls would further increase the number of responses obtained but, in this study, no such effect was apparent. This may be because only individuals that regularly utilised the area, near to which the stimulus calls were broadcast, may have been motivated to respond to the stimulus whereas, individuals further away that may have heard the amplified call where less likely to alter their behaviour. In many species, individuals compete for resources but avoid escalated conflicts by using threat displays that allow the opponent to predict the outcome of the conflict without the necessity of fighting (Bradbury and Vehrencamp, 1998; Maynard-Smith and Harper, 2003). A study of male sac-winged bats *S. bilineata* found that male territorial holders can adjust their vocal territorial displays according to the peak frequency of the territorial songs of their opponents (Behr et al., 2009). Playback experiments with territorial males found that low frequency stimuli elicited a higher territorial song rate and length than high frequency stimuli. It has been shown that male *S. bilineata* that sing more often with lower peak frequencies sire more offspring than their competitors (Behr et al., 2006), and the

peak frequency of territorial calls may indicate male quality and the resulting threat posed to competitors.

Loud, long-distance vocal signals can serve a number of different functions simultaneously. Song in male birds, for example, not only functions in territorial defence, but is also used to attract mates and can advance female ovulation (Catchpole and Slater, 1995). Similarly, some male mammals have loud calls that serve multiple functions within the context of reproduction and indeed these functions can be entirely compatible if the signaller is both ready to engage in male–male competition and to mate with females (e.g. red deer *Cervus elaphus*) (Clutton-Brock and Albon, 1979). In this study it may be that the observed increased response rate for high intensity high frequency calls is a result of a number of different causal factors, which may interact in unison.

There was also evidence of seasonal variation in the response to each call type. Earlier in the season loud, high repetition rate calls were significantly more effective whereas later in the season quiet calls were just as likely to elicit an approach response. In turn, later in the season, quiet, high repetition rate calls were significantly more likely to elicit an approach response than quiet low calls. Many studies have found differences in social call production that relates to mating. For example, studies of the social calls of the common pipistrelle bat *Pipistrellus pipistrellus* found that the production of songflight calls occurred more frequently in the mating season (August – September) (Russ et al., 2003; Budenz et al., 2009), and social calls recorded at hibernation sites of greater horseshoe bats *Rhinolophus ferrumequinum* in late October were inferred by the authors to be mating calls, as these calls were absent from summer roosting sites (Andrews et al., 2006). This assumption, however, is speculative as social calls produced at hibernacula may have other functions such as information transfer (Wilkinson, 1995). To date no studies have investigated the effects of

season on the responses to social calls of differing intensity. This study has demonstrated that loud, repetitive calls elicit stronger responses earlier in the season compared to later on in the season, which could mean that loud repetitive calls could be used as defence of resources at a critical time early in the season or for attracting conspecifics to a rich resource to enable exploitation.

In addition to the effect of seasonal differences in responses in relation to call intensity, there was also evidence of an association between call type and observed behaviour. Loud calls with high repetition calls (such as call A) were significantly more frequently associated with a response assigned to behaviour categories that were longer in duration and more complex, whereas quiet calls (such as B and C) were more frequently associated with shorter duration responses. However, it is not possible to deduce from these patterns the functions of these differing intensity calls types as, in this scenario, the identity of the responding individuals was not known in most instances. There was no significant difference in the composition of responses to different call types between known females and the remaining responses where the sex of the bat was not know. However, the general trend does allow for the formulation of hypotheses, for example, do high intensity calls elicit more prolonged responses for females earlier in the season as a function of resource defence? This hypothesis could subsequently be tested under different conditions, such as a smaller woodland with all of the females radio-tagged and marked with reflective tape.

Social calls were recorded at the same time as 51% of the filmed behavioural responses of *P. auritus*. Although there is the possibility that these call(s) were not produced by the responding bat(s), this is extremely unlikely given that *P. auritus* social calls are recorded so infrequently under natural conditions. This supports the hypothesis that responses to the

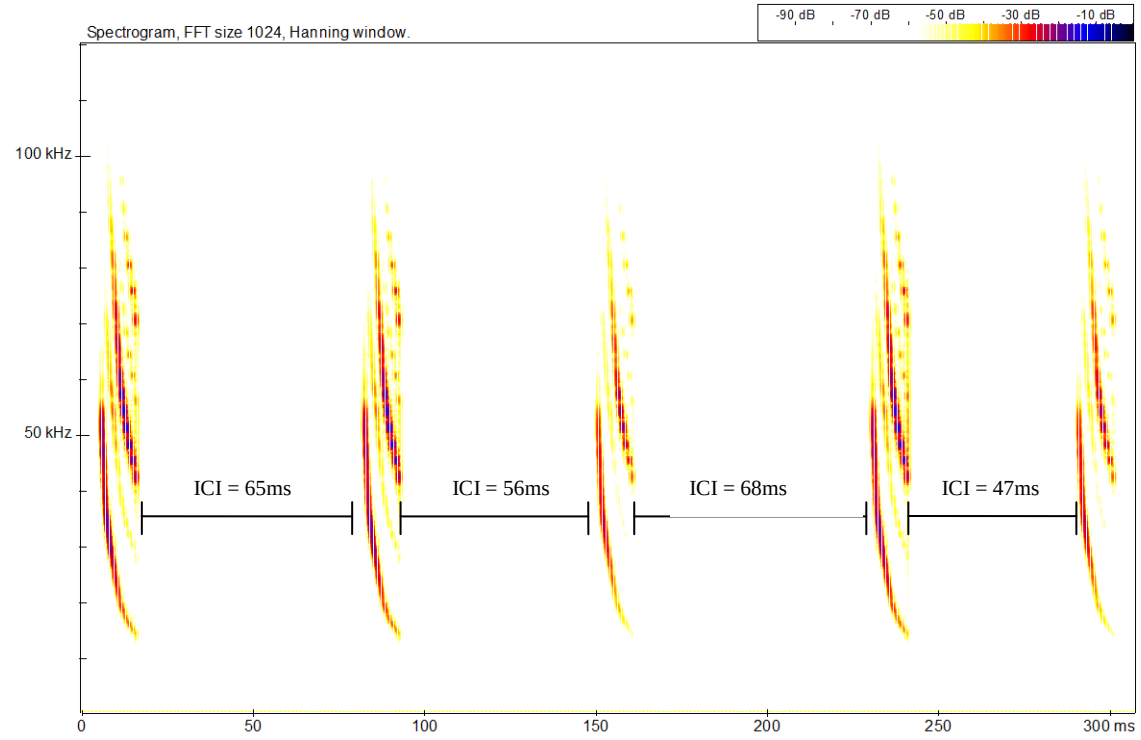
stimulus represent a social response as opposed to simply an investigation of a novel sound source. Social call production was significantly associated with the production of the stimulus which suggests that the bats were vocally responding to the stimulus. Many bat species use specific vocalisations in a given behavioural situation (Barclay and Thomas, 1979; Pfalzer and Kusch, 2003; Davidson and Wilkinson, 2004), and these vocalisations have been shown to convey information about both individual (Fenton et al., 2004) and group identity (Boughman and Wilkinson, 1998). A perception of identity (Balcombe and McCracken, 1992; deFanis and Jones, 1996) and group association (Boughman and Wilkinson, 1998) has, in addition, been reported for some bat species.

4.6 - General Discussion

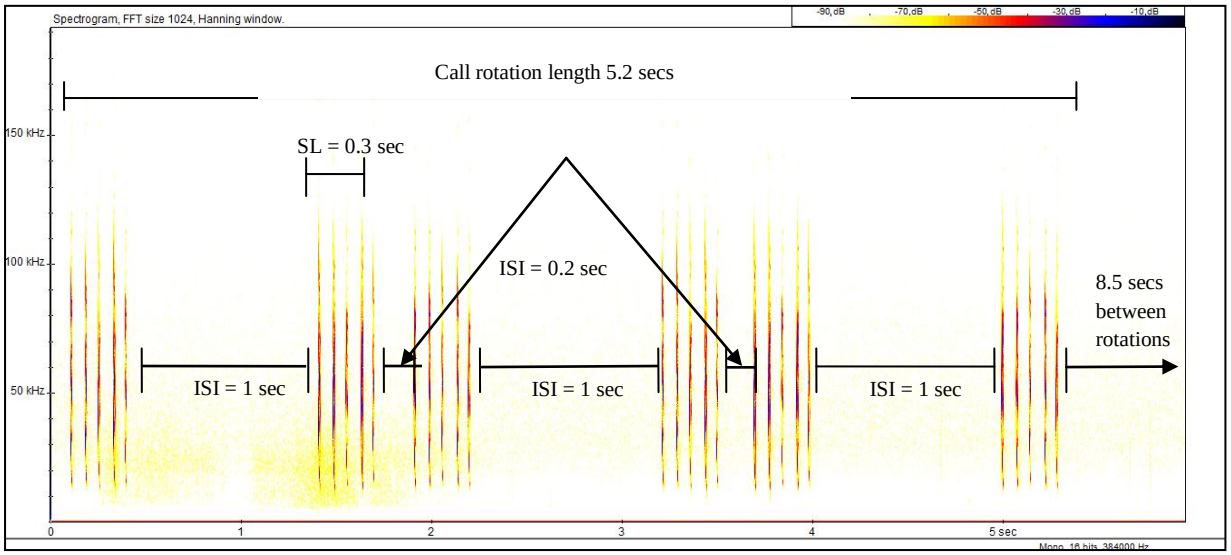
One explanation for the females' response to the Autobat may be that it represents an investigation of, and an attempt to expel, an unfamiliar bat detected within the home range. Defence of a feeding area may be more critical during late pregnancy and lactation, when energy demands on the females are highest. This may help to explain why female responses (at least as reflected by capture rates) declined in the latter half of the summer. One way of investigating a possible home range defence role of responses to social calls is to compare the responsiveness of females in relation to whether the stimulus is located within or outside of their ranging area. However, to determine whether the context the call was produced in is important, knowledge pertaining to the home range of the focal individual, and the distance that they are likely to respond to the stimuli, must be undertaken.

4.7 - Summary

- There were significantly more responses filmed, per hour, when the Autobat was broadcasting social calls than when the Autobat was silent.
- *P. auritus* were clearly much more responsive to simulations of their own species' social calls than to the other stimuli tested
- This chapter has shown that responses vary in relation to differences in the stimulus, and, also, seasonally. Although responses by banded individuals were too few to allow a detailed analysis of sex differences in response, the seasonal change in captures suggests that the function of the calls varies seasonally, with males becoming more responsive as the mating season begins.



Sonogram 1



Sonogram 2

Figure 4.1: Simulated *P. auritus* social call, 'stimulus A' was comprised of a sequence of five descending FM sweeps as shown in sonogram 1. These were played as a series of sequences at irregular intervals as shown in sonogram 2. The entire length of the stimulus rotation is 5.2 seconds. This was followed by an interval of 8.5 seconds of silence before the stimulus rotation was repeated again. ICI = inter-call interval (time). ISI = inter-sequence interval (time). SL = sequence length (time).

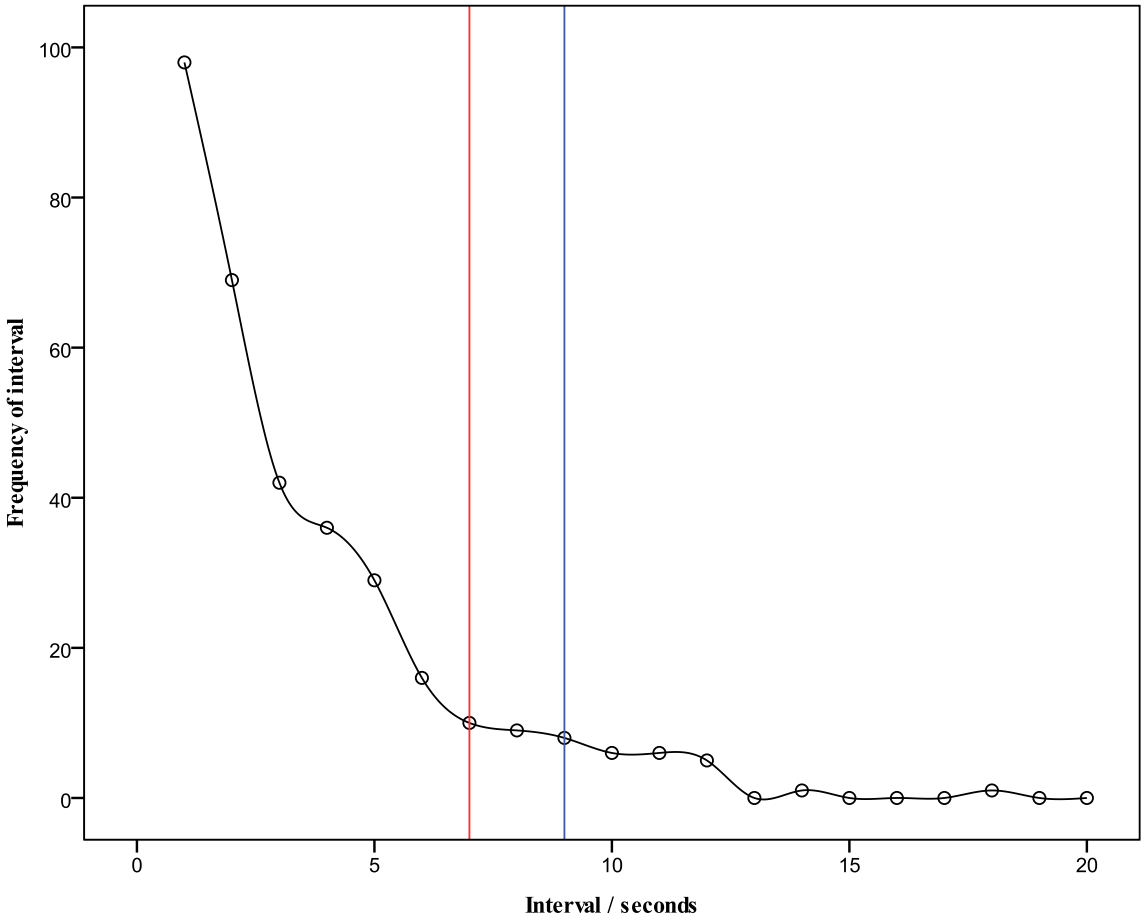


Figure 4.2: The frequency of observations of bats recorded on video was plotted against the interval between successive observations in order to obtain a suitable interval between observations to edit the film into separate 'responses'.

Figure 4.3: This photograph shows that the bat was within 12.5 cm of the box in the vertical plane, but it is not possible to tell how far it was in the horizontal plane.

Figure 4.4: This photograph shows that the same bat was within 25 cm of the box in the horizontal plane.

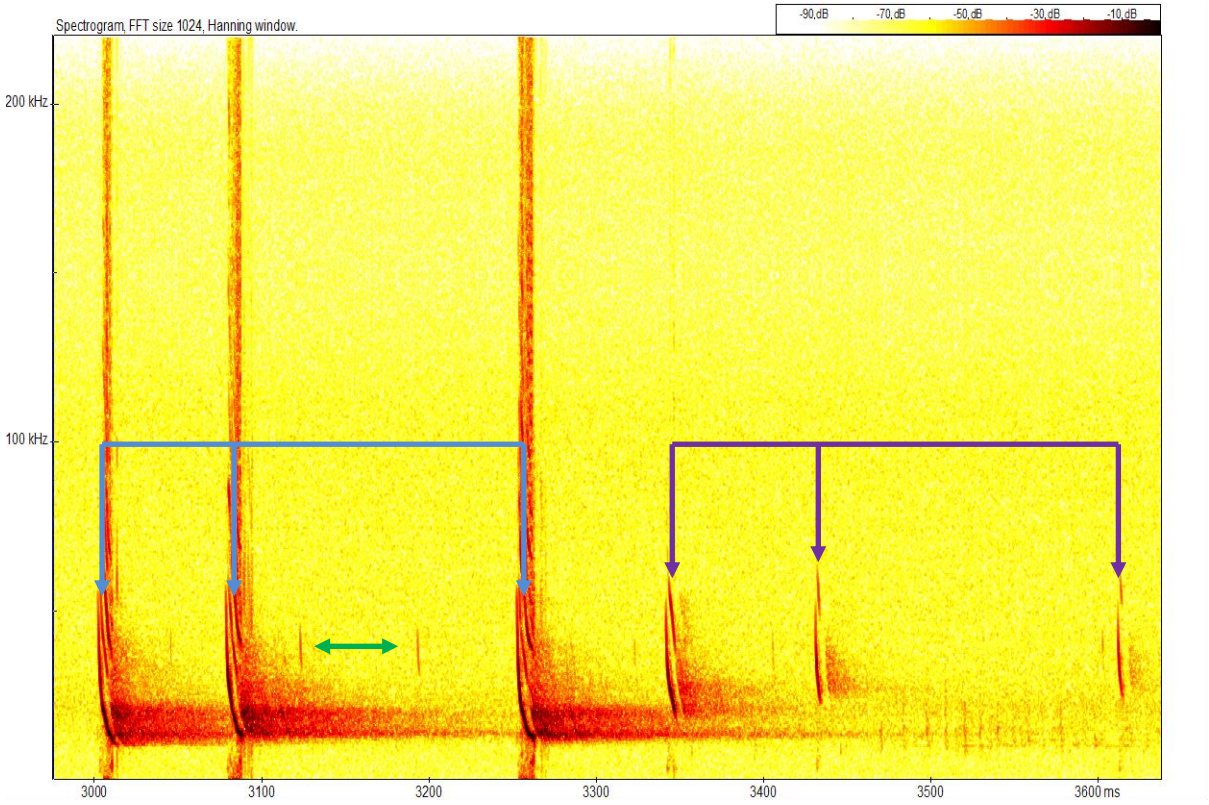


Figure 4.7: A *Plecotus auritus* response to synthesised social calls produced by the Autobat. The simulated social calls are indicated by the blue arrows. The responding *Plecotus auritus* initially echolocates between the simulation broadcast, as indicated by the green arrows, but three clear social calls are produced at the end of the sequence, as indicated by the purple arrows.

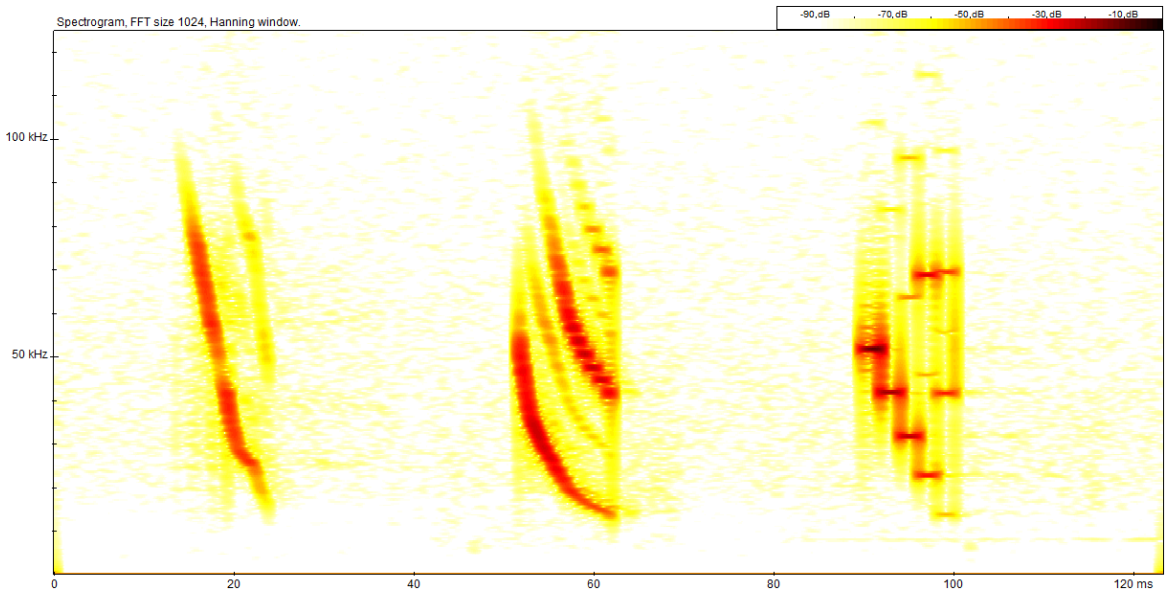
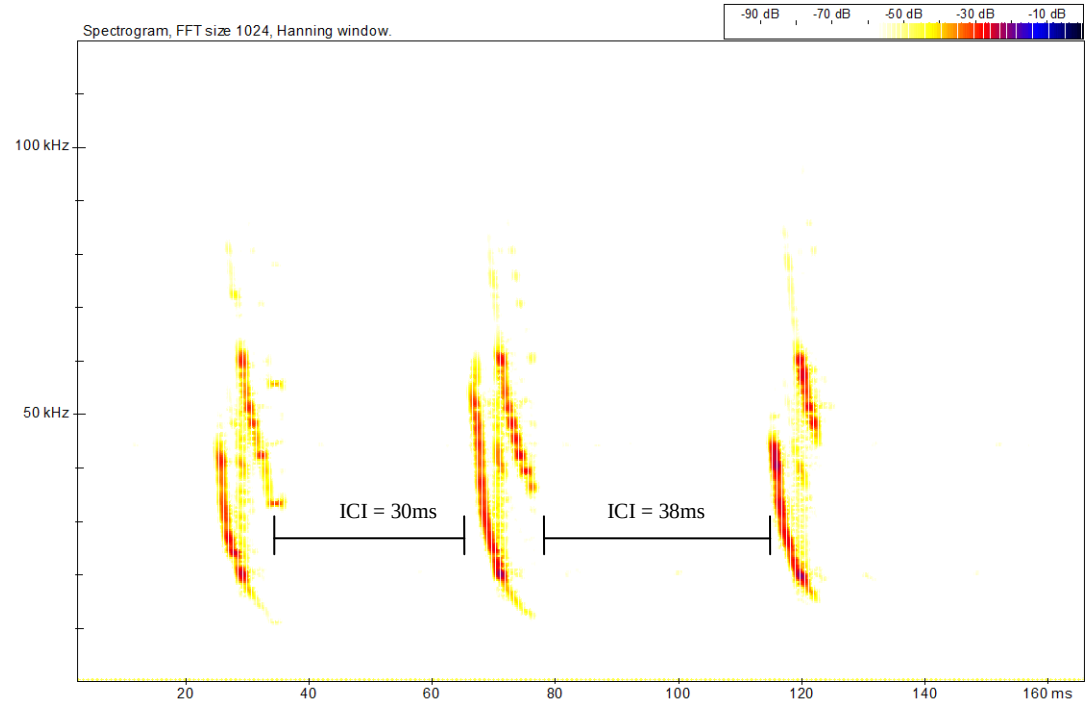
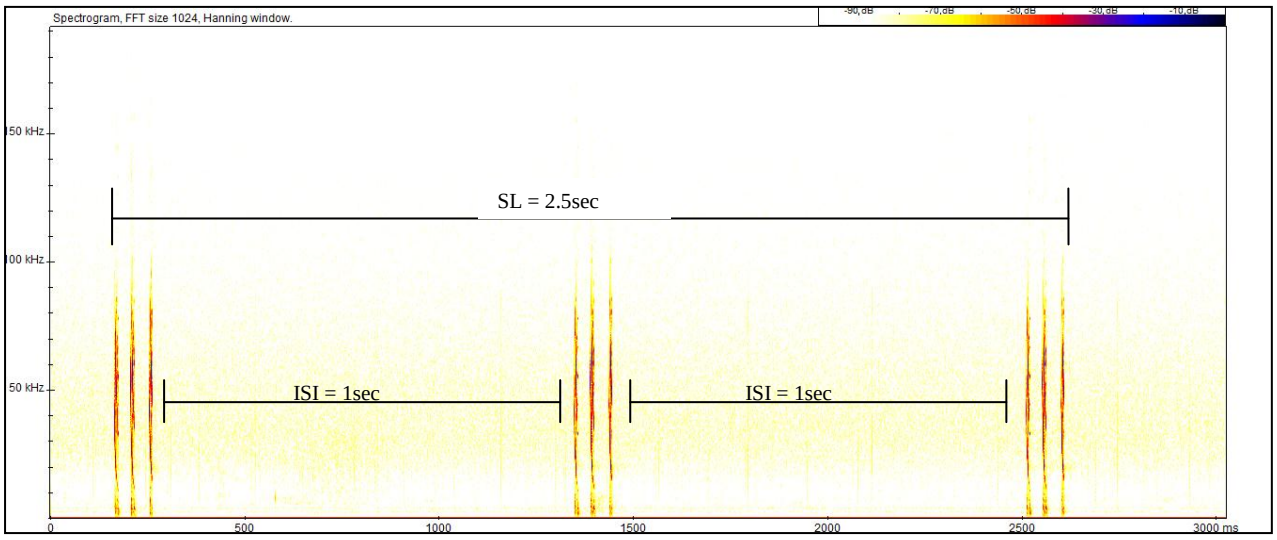


Figure 4.8: The first sweep is a simulated Bechstein's bat social call; the second is a simulated Brown long-eared bat social call and the third is a series of five brief, descending tones that follow a similar trajectory to the brown long-eared call. Each sweep is between 11 and 12 milliseconds in duration. Each was played in an irregular sequence of five sweeps, following the same temporal pattern for each call, with the same intersequence interval.

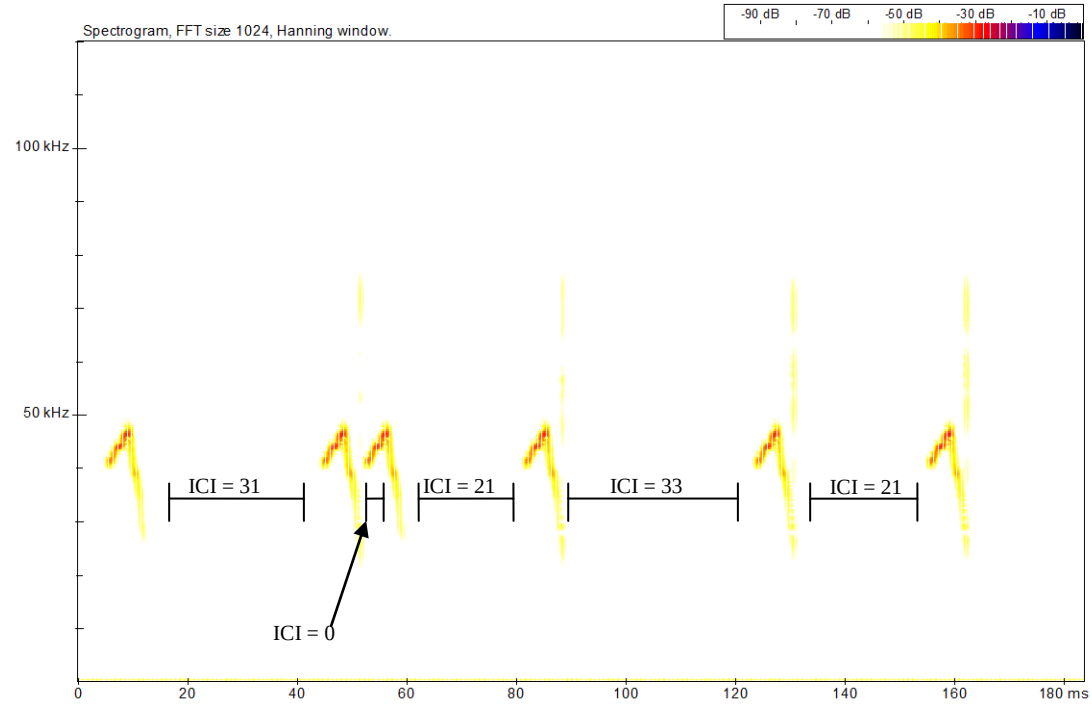


Sonogram 1

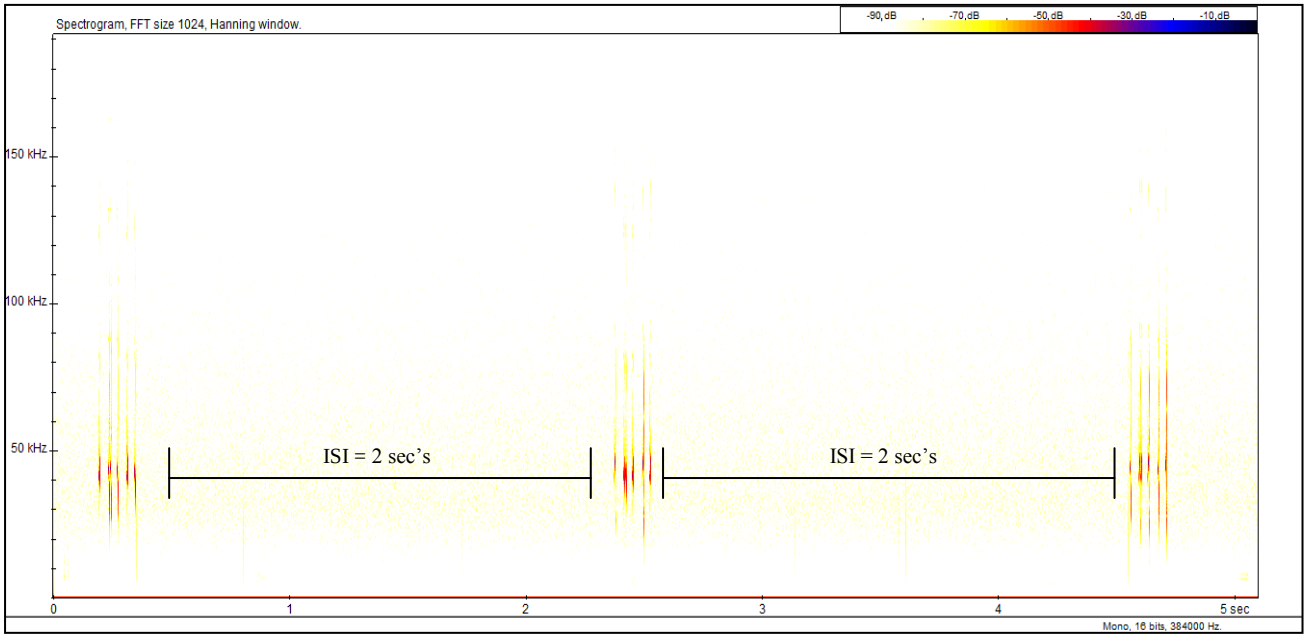


Sonogram 2

Figure 4.11: Simulated *Plecotus auritus* social call, 'stimulus B' was comprised of a sequence of three descending FM sweeps as shown in the sonogram 1. These three sweeps were played as a series of three sequences at one second intervals, as shown in Sonogram 2. The entire length of the stimulus rotation is 2.5 seconds. This was followed by an interval of 9.5 seconds of silence before the stimulus rotation was repeated again. ICI = inter-call interval (time). ISI = inter-sequence interval (time). SL = sequence length (time).

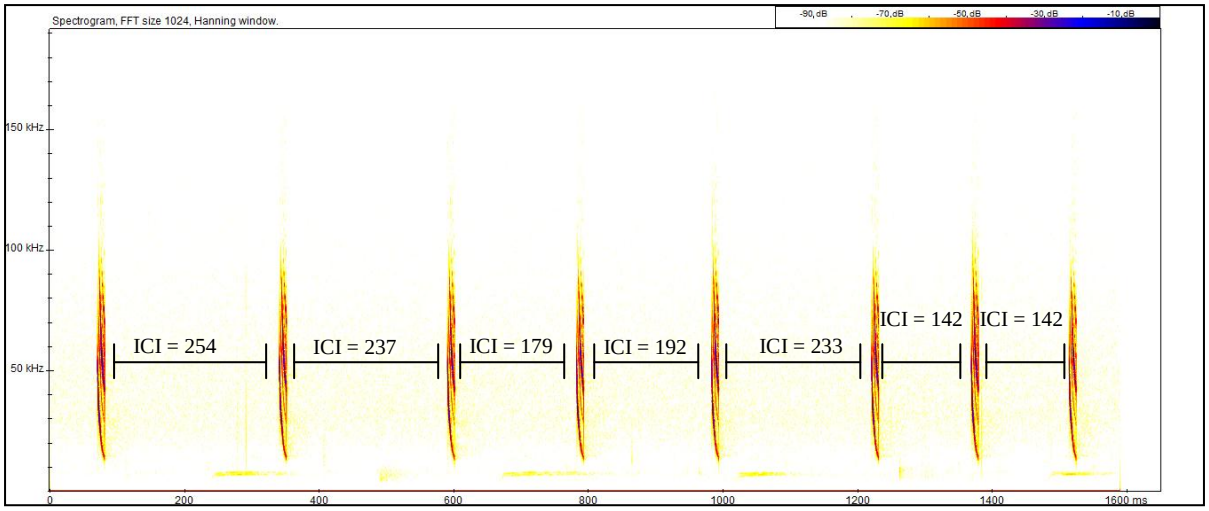


Sonogram 1

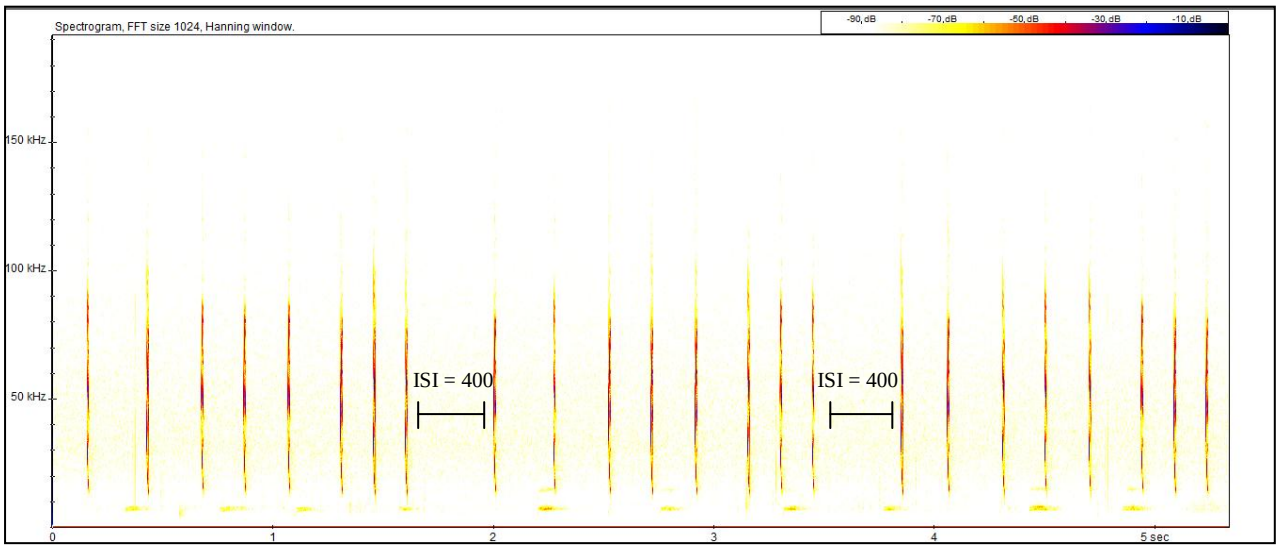


Sonogram 2

Figure 4.12: Simulated *Plecotus auritus* social call, 'stimulus C' was comprised of a six FM sweeps that ascended and descended shown in sonogram 1. The sequence lasted for a total of 160ms with an interval of two seconds between each sequence before the sequence is repeated again. ICI = intercall interval (time). ISI = inter-sequence interval (time).

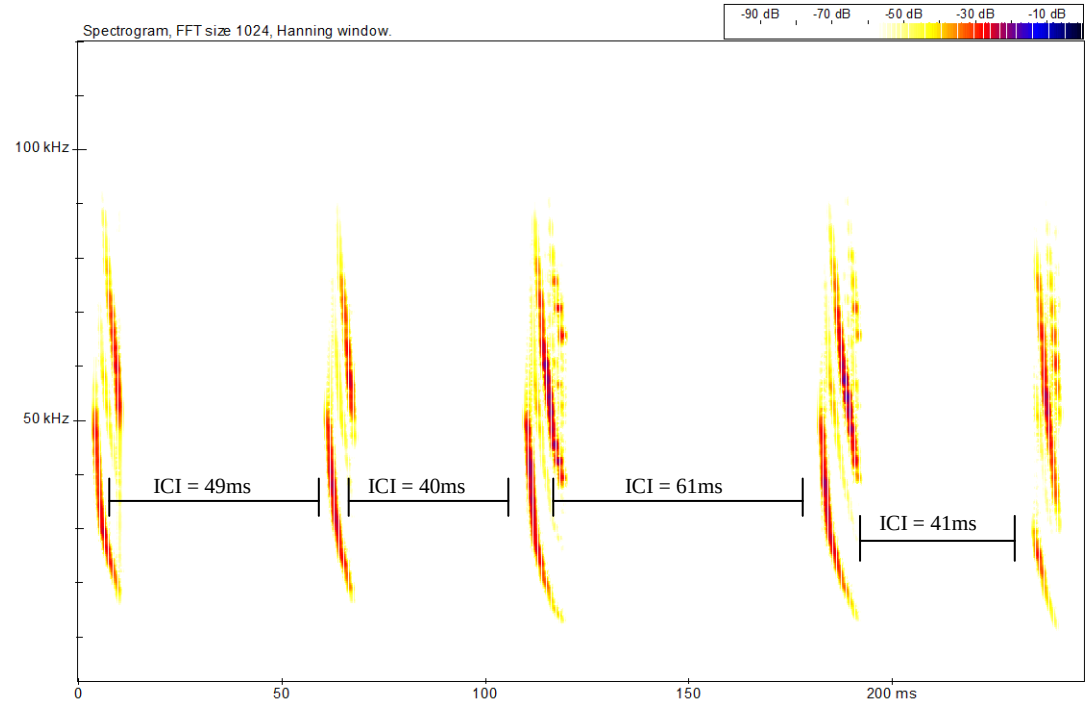


Sonogram 1

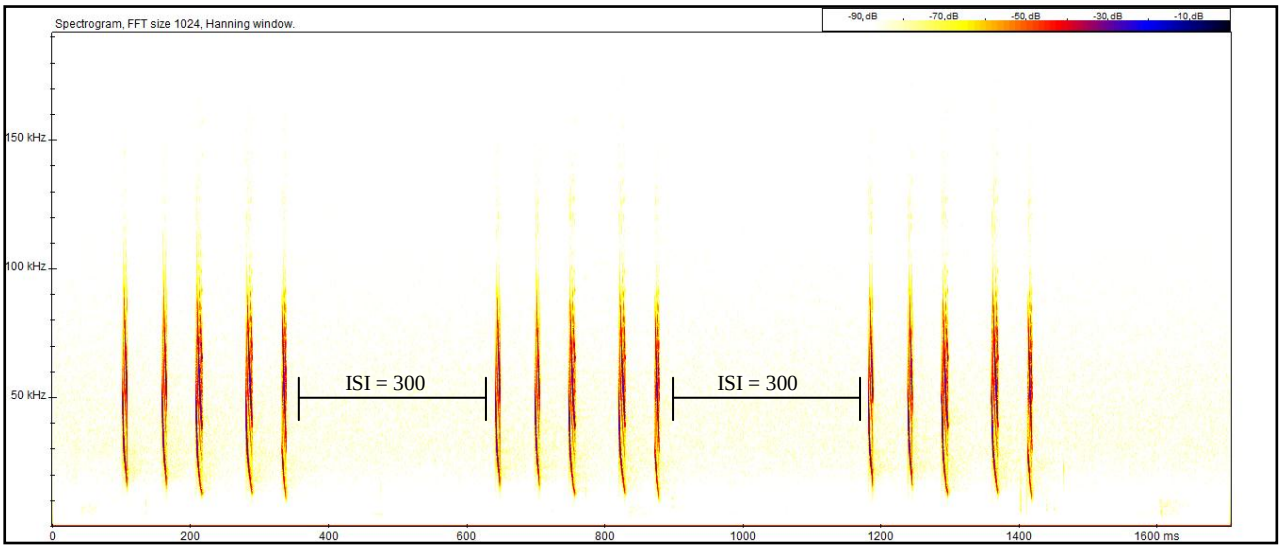


Sonogram 2

Figure 4.13: Simulated *Plecotus auritus* social call, 'stimulus D ' was comprised of a sequence of eight descending FM sweeps as shown in sonogram 1. These eight sweeps were played as a series of ten sequences, with an interval of 0.4 seconds, as shown in sonogram 2 (only three of the ten sequences shown). The entire length of the sequence rotation is 18 seconds. This was followed by an interval of 12.5 seconds of silence before the call rotation was repeated again. ICI = inter-call interval (time). ISI = inter-sequence interval (time). SL = sequence length (time).

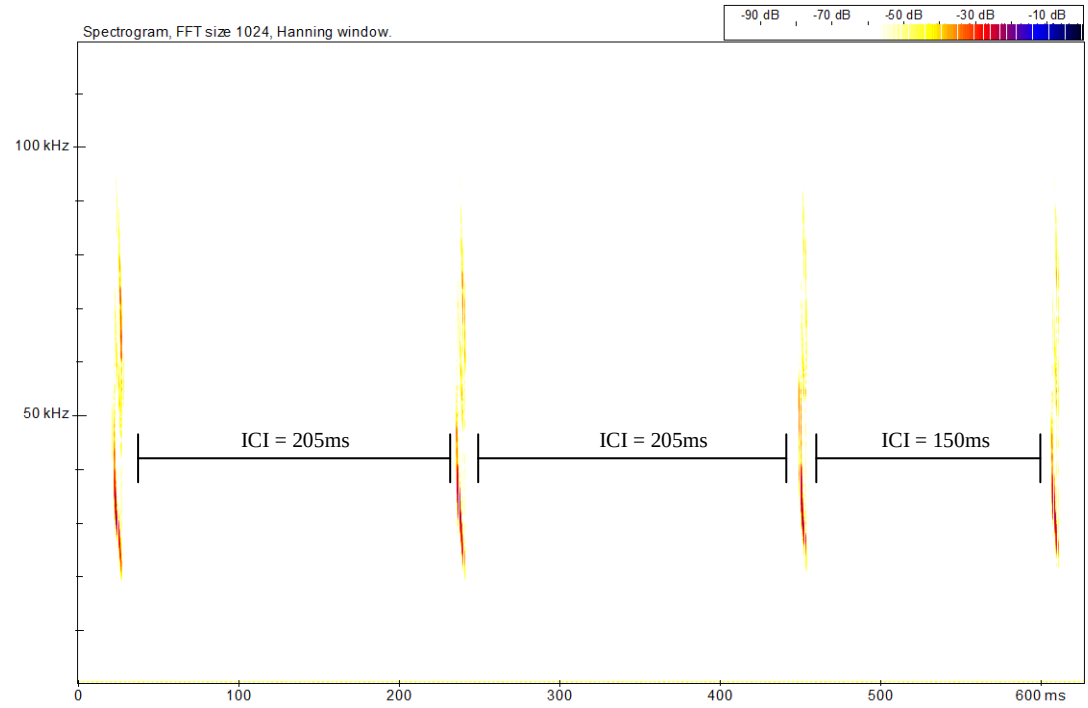


Sonogram 1

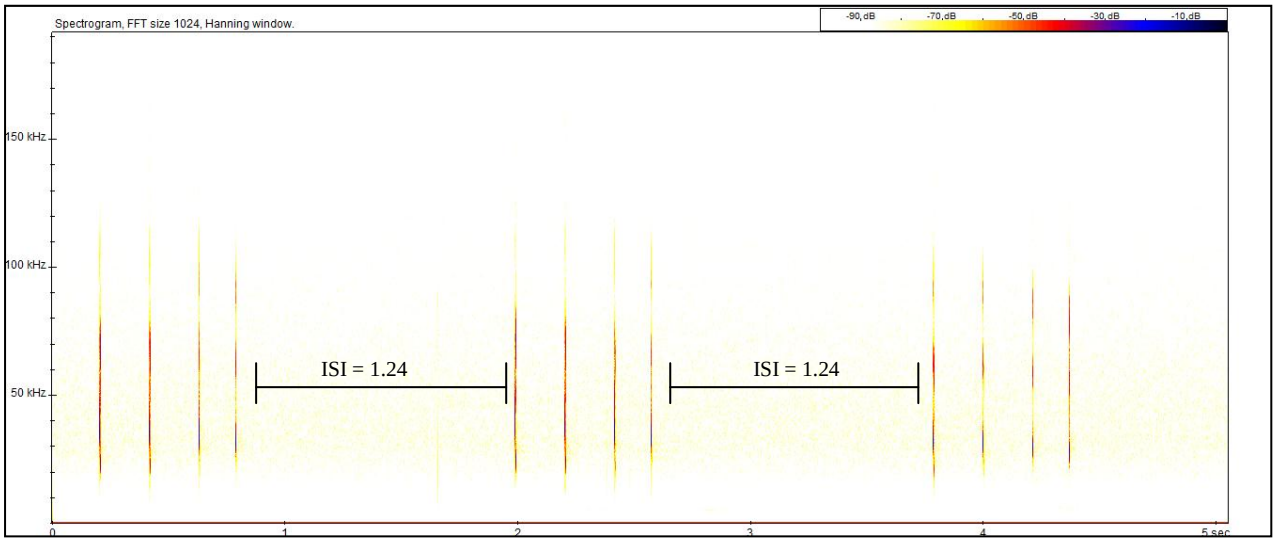


Sonogram 2

Figure 4.14: Simulated *Plecotus auritus* social call, 'stimulus E' was comprised of a sequence of five descending FM sweeps as shown in sonogram 1. These five sweeps were played as a series of three sequences at 0.13 second intervals. The entire length of the call rotation is 0.13 seconds. This was followed by an interval of 6.7 seconds of silence before the stimulus rotation was repeated again. ICI = inter-call interval (time). ISI = inter-sequence interval (time).



Sonogram 1



Sonogram 2

Figure 4.15: Simulated *Plecotus auritus* social call, 'stimulus F' was comprised of a basic sequence of four descending FM sweeps as shown in sonogram 1. These four sweeps were played as a series of nine sequences at intervals of 1.24 seconds, (three sequences are shown in sonogram 2). The entire length of the call rotation is 16.5 seconds. This was followed by an interval of 12.5 seconds of silence before the call rotation was repeated again. ICI = intercall interval (time). ISI = Intersequence interval (time = seconds).

	A	B	C	D	E	F
No of sweeps per sequence	5	3	6	8	5	4
No of sequences in rotation	6	3	n/a	10	3	9
No of sequences / min	26.28	15	27.77	19.67	7.47	18.63
No of sweeps / min	131.4	45	166.66	157.37	112	74.52
Number of sweep types / sequence	1	3	2	1	3	1
Sweep type 1: Duration(ms)	12	12	8	12	9.1	8
Sweep type 1: Fmin(kHz)	13.7	11.1	25	13.2	14	20.6
Sweep type 1: Fmax(kHz)	56.3	50.9	51.2	67	60	58.5
Sweep type 1: Fmax(e)(kHz)	32.7	20.7	47	48.0	34	32
Sweep type 1: dB SPL @ Fmax(e)	51.5	43.0	38.7	53.8	44.0	48.7
Sweep type 2: Duration(ms)	n/a	11.5	8.9	n/a	12	n/a
Sweep type 2: Fmin(kHz)	n/a	12	19.9	n/a	12.5	n/a
Sweep type 2: Fmax(kHz)	n/a	69	49.7	n/a	61	n/a
Sweep type 2: Fmax(e)(kHz)	n/a	20.7	44.9	n/a	42.0	n/a
Sweep type 2: dB SPL @ Fmax(e)	n/a	42.0	37.0	n/a	49.5	n/a
Sweep type 3: Duration(ms)	n/a	9.3	n/a	n/a	7.8	n/a
Sweep type 3: Fmin(kHz)	n/a	14.9	n/a	n/a	10	n/a
Sweep type 3: Fmax(kHz)	n/a	48.7	n/a	n/a	33.7	n/a
Sweep type 3: Fmax(e)(kHz)	n/a	20.1	n/a	n/a	25.0	n/a
Sweep type 3: dB SPL @ Fmax(e)	n/a	42.3	n/a	n/a	37.1	n/a

Table 4.3: Acoustic parameters of the six stimuli A – F. Stimuli A, D, & F has only one sweep type per sequence (hence only one set of measurements for each stimuli). Stimulus C had two different types of sweep per sequence and stimuli B & E had three different types of sweep per sequence.

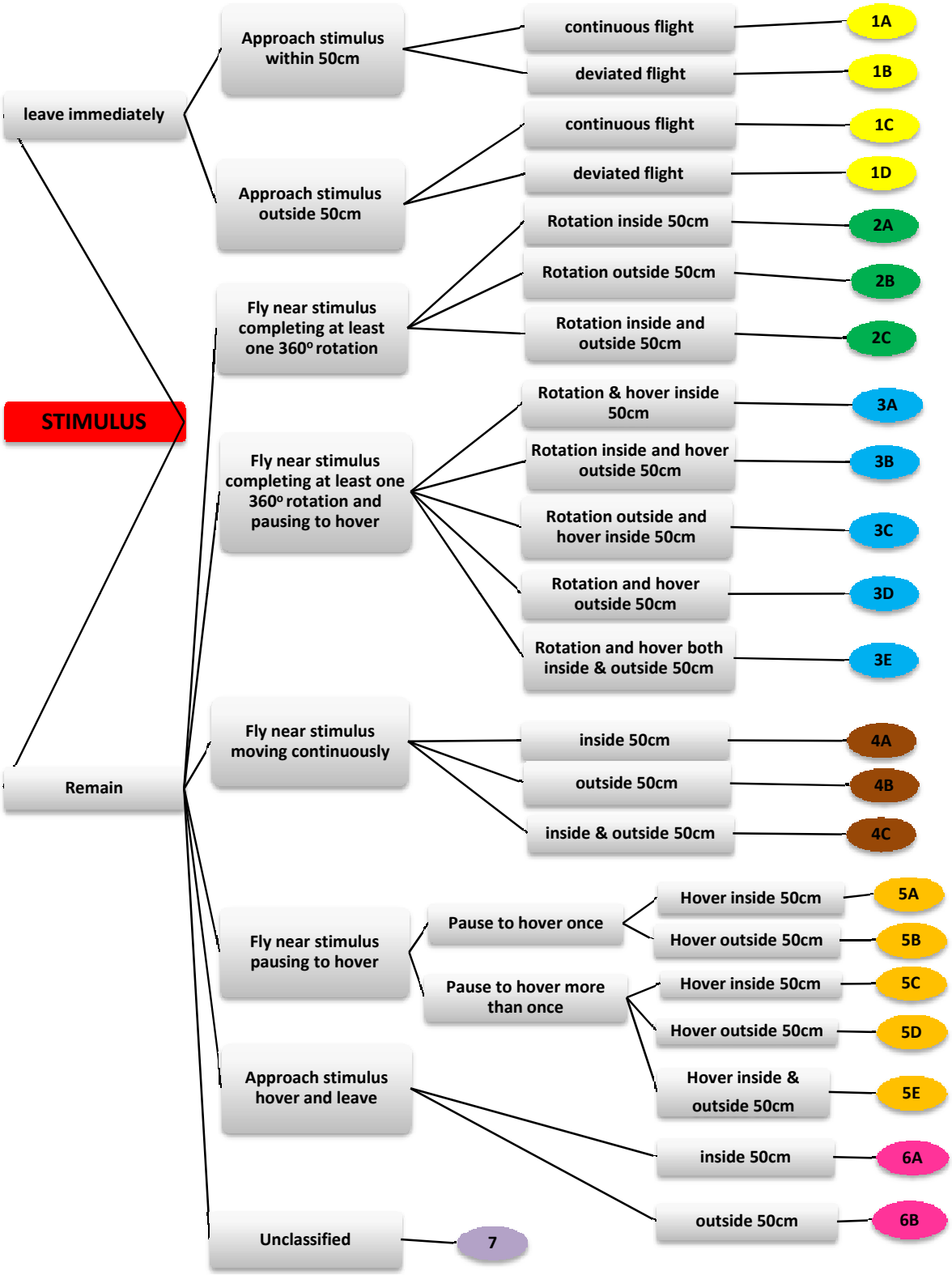


Figure 4.16: Categorisation of *Plecotus auritus* behavioural responses to simulated social calls.

Source of variation	SS	SS/MS total	d.f.	*P*-value
Month	34.438	23.58	4	0.0002
Response Type	59.573	40.80	6	0.0001
Month*Response Type interaction	88.600	63.28	24	0.0010

Table 4.5: The results of the Scheirer-Ray-Hare test showed that there are significant differences in relation to month, response type and a significant effect of an interaction for both month and response type.

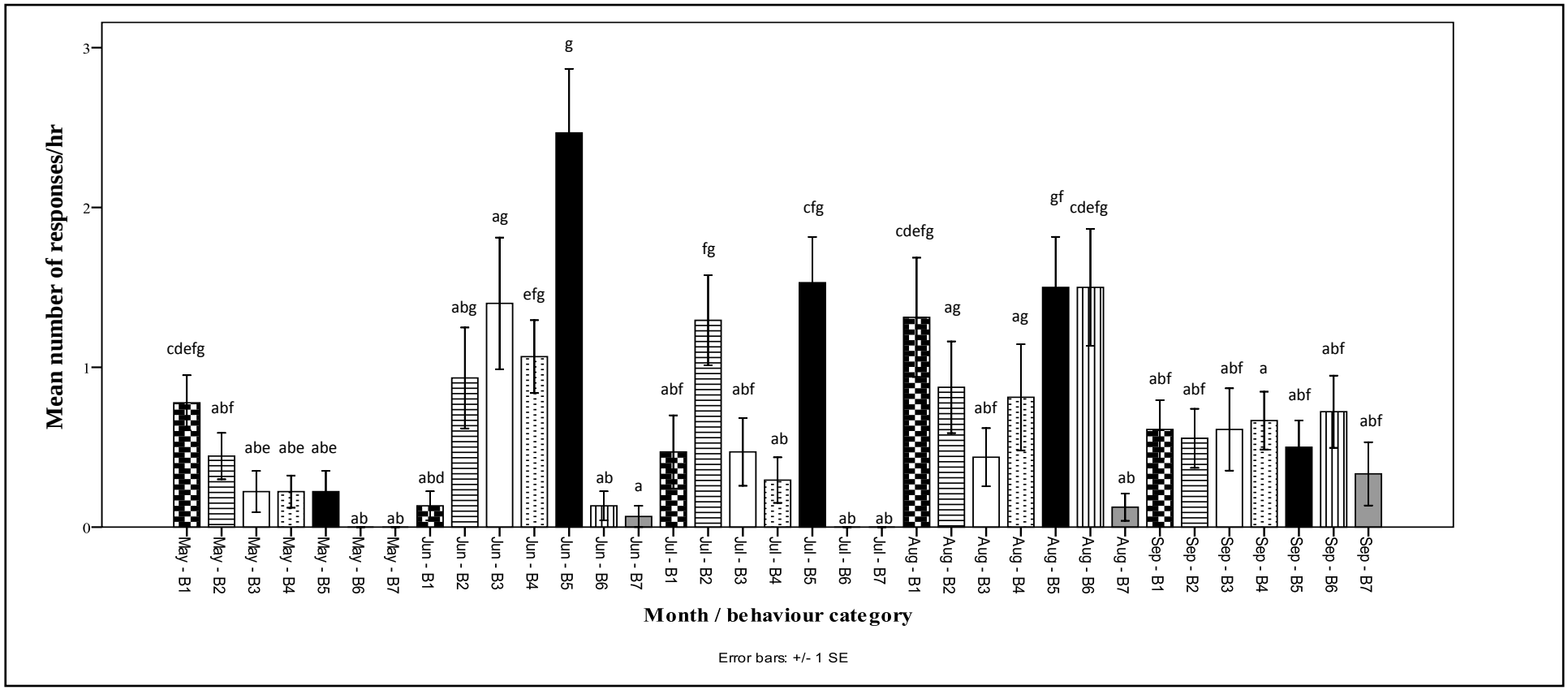


Figure 4.20: Mean number of responses for each behaviour category in each month. Where the letters are different it indicates a significant difference between groups.

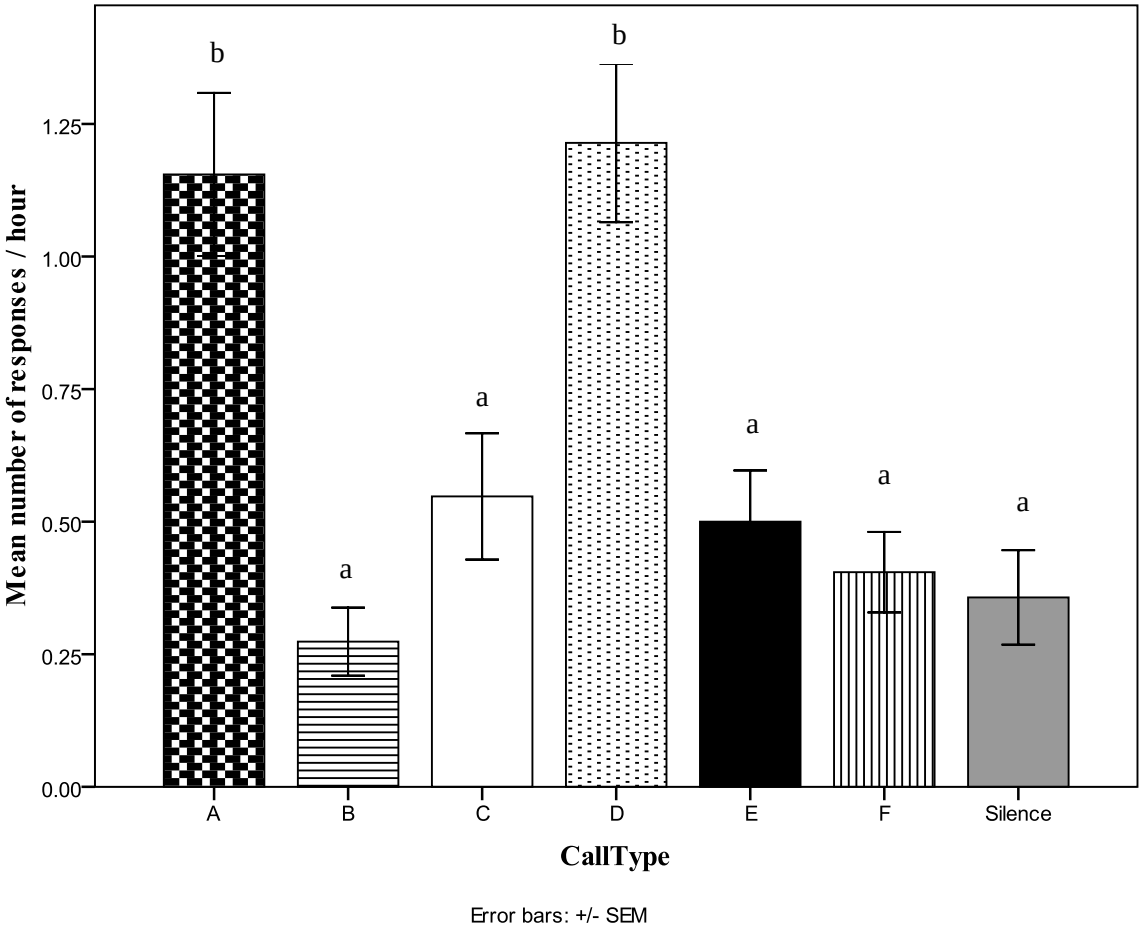


Figure 4.21: Total number of *P. auritus* responses to each stimuli type recorded over the 172 hours of filming. Both stimuli A and call D, which have a high intensity and repetition rate, elicited responses more frequently compared to, for example, call B, which has a low intensity and low repetition rate. Where the letters are different it indicates a significant difference between groups.

Source of variation	SS	SS/MS total	d.f.	*P*-value
Month	34.682	22.09	4	0.001
Call type	81.718	52.04	6	0.001
Month*Call Type interaction	99.266	63.47	24	0.001

Table 4.7: The results of the Scheirer-Ray-Hare test showed that there are significant differences in relation to month, call type and a significant effect of an interaction for both month and call type.

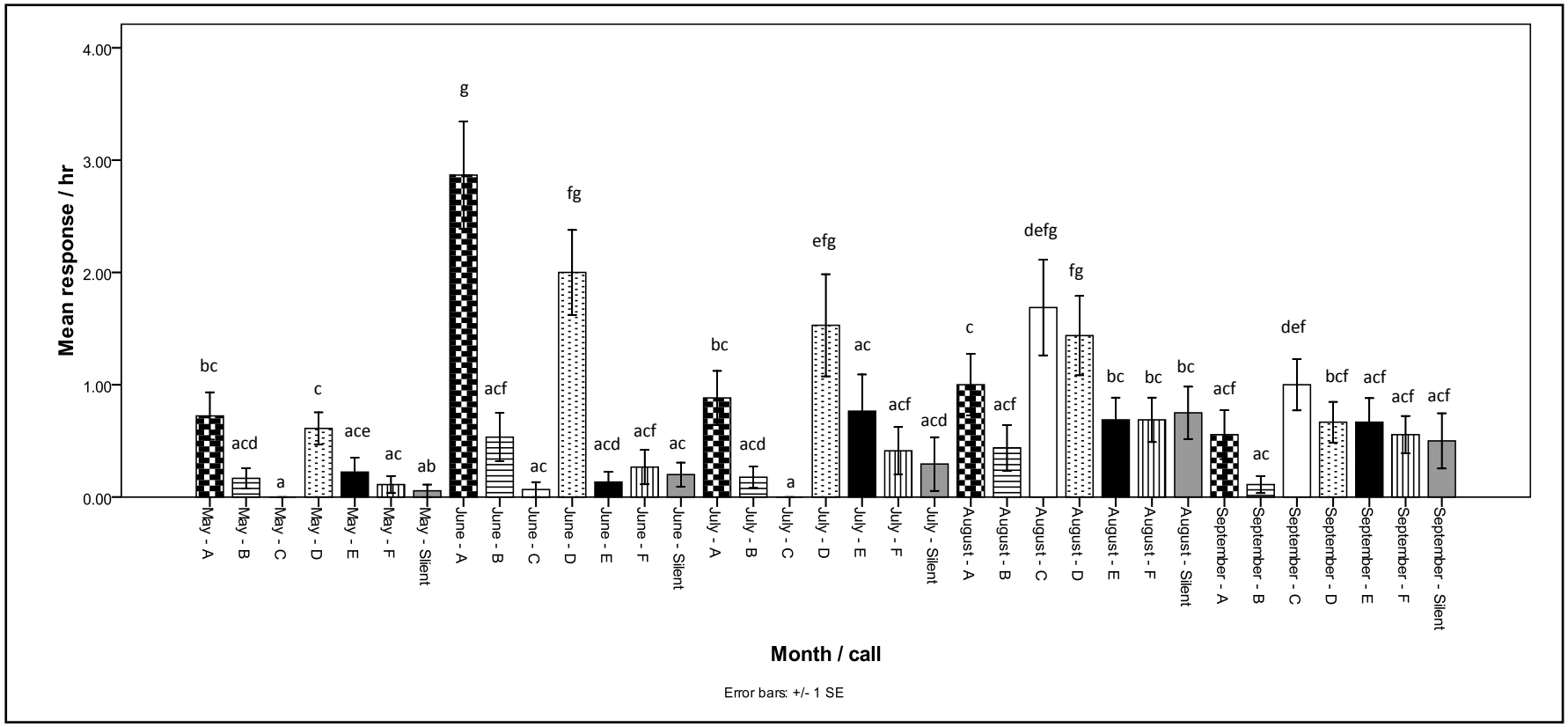


Figure 4.22: Mean number of responses for each call type in each month. Where the letters are different it indicates a significant difference between group means.

Call	**Test Statistic**	***n***	***p value***
A	Z = - 0.191	20	0.848
B	Z = - 0.191	20	0.848
C	Z = - 0.773	20	0.440
D	Z = - 0.038	20	0.969
E	Z = - 0.077	20	0.939
F	Z = - 0.824	20	0.410

Table 4.8: Effect of amplification on the number of responses for each simulated social call. There were no significant differences in the number of responses by amplification of each call type.

	No. of responses where approach was within 50cm of stimulus	**No. of responses where approach was outside 50cm of stimulus**	**Totals**
Call without amplification	133	38	171
Call with amplification	114	63	177
Totals	247	101	348

Table 4.9: There was a significant association between amplified calls and proximity of overall approach.

call * behaviour _category crosstabulation

			Behaviour category						
			1	2	3	4	5	6	Total
call	A	Count	6	14	21	11	45	1	98
		Expected Count	16.8	16.2	14.5	11.7	27.6	11.1	98
		Std. Residual	-2.6	-.6	1.7	-.2	**3.3**	-3.0	
	B	Count	12	6	2	5	0	0	25
		Expected Count	4.3	4.1	3.7	3.0	7.0	2.8	25
		Std. Residual	**3.7**	.9	-.9	1.2	-2.7	-1.7	
	C	Count	0	1	2	4	5	29	41
		Expected Count	7.0	6.8	6.1	4.9	11.6	4.6	41
		Std. Residual	-2.7	-2.2	-1.7	-.4	-1.9	**11.3**	
	D	Count	27	28	2	2	40	2	101
		Expected Count	17.3	16.7	15.0	12.0	28.5	11.5	101
		Std. Residual	2.3	2.8	**-3.4**	-2.9	2.2	-2.8	
	E	Count	10	2	15	14	5	2	48
		Expected Count	8.2	8.0	7.1	5.7	13.5	5.4	48
		Std. Residual	.6	-2.1	3.0	3.1	-2.3	-1.5	
	F	Count	4	6	9	5	2	5	31
		Expected Count	5.3	5.1	4.6	3.7	8.7	3.5	31
		Std. Residual	-.6	.4	2.1	.7	-2.3	.8	
Total		Count	59	57	51	41	97	39	344
		Expected Count	59	57	51	41	97	39	344

Table 4.10: Results of Chi Sq test of association. Standardised residuals that have a positive value mean that the cell was over-represented in the actual sample, compared to the expected frequency. Standardised residuals that have a negative value mean that the cell was under-represented in the actual sample, compared to the expected frequency. Using a critical value of Z = +/- 3.22, there are 3 positive (highlighted in yellow) and 1 negative (in red font) associations between call and observed behavior category.

5.1 – Introduction

Field experiments with infra-red video demonstrated that brown long-eared bats, *Plecotus auritus*, responded to synthesised social calls by rapidly approaching the source (Chapter 4). One hypothesis for the response of *P. auritus* to synthesised social calls at foraging sites is that the responses are related to home range use and represent defence of resources. In order to examine this hypothesis, it was necessary to establish the extent of home range and determine activity behavioural patterns.

'Telemetry' was defined by (Priede, 1992) as any method of obtaining information on living free-ranging animals by remote means. The attachment of radio-transmitters to animals allows the emitted signal to be located on subsequent occasions and, from this, the animal's movements can be inferred (Kenward, 1987). Radio-telemetry has become the method of choice for studying movement patterns and activity of many nocturnal mammals, including bats (Wilkinson and Bradbury, 1988). The benefits of radio-telemetry for studying bats include: (i) the locations of individual bats can be monitored over considerable distances, often for a period of weeks; (ii) direct observations of individuals during the day (i.e. day roost locations) and, potentially, during the night are possible because the bats location(s) can be determined (Wilkinson and Bradbury, 1988).

Most animals use the same area repeatedly over time (Darwin, 1861), hence animal movements are often defined using the home range concept (Jetz et al., 2004; Anich et al., 2009) where the home range is the area used by an animal over a given time interval (Burt, 1943; White and Garrott, 1990). Animals are expected to utilise home ranges that represent

the minimum economically defensible area (Pyke et al., 1977; Maher and Lott, 1995; Borger et al., 2008) but which are large enough to provide food resources and shelter over time (McNab, 1963; Reiss, 1988; Dammhahn and Kappeler, 2009). Some studies have proposed that the inter-specific variation in home range is typically correlated with body size (i.e. larger animals have larger home ranges) (McNab, 1963; Reiss, 1988; Dutoit, 1990). However, the size of an animal's home range may also vary with sex (Borger et al., 2006), season (Wiktander et al., 2001), age (Cederlund and Sand, 1994) and, also, population density (Damuth, 1981) both intra- and inter- specifically.

Radio-tracking studies of Microchiropteran bats have revealed inter- and intra-specific variation in home ranges. Range sizes for barbastelle *Barbastella barbastellus*, for example, have been reported to be in the region of 125 to 2551 ha (median:403ha for 12 radio-tracked females) (Hillen et al., 2009) whereas reported range sizes for Bechstein's *Myotis bechsteinii* females are substantially smaller, 9.9ha to 37.5ha (mean 20.4ha for 10 radio-tracked females (Kerth et al., 2001). Considerable variation in range size has also been reported for morphologically similar species. For example, radio-tracking studies on two species of sympatric pipistrelle bats in the UK *Pipistrellus pipistrellus* and *Pipistrellus pygmaeus* have revealed that *P.pipistrellus* home ranges were on average three times larger than those of *P. pygmaeus* (Nicholls and Racey, 2006) and there were also considerable differences in range size of individuals within species (Davidson-Watts and Jones, 2006). These reported variations in range sizes do not support the hypothesis that range size is related to body mass, and alternative explanations, such as the distribution of resources (e.g. prey) in a heterogeneous environment, may better explain variation in range size (Carr and Macdonald, 1986). Such patchy distribution of prey may mean that the resource defence hypothesis is not appropriate for some species of bat (Hickey and Fenton, 1990).

P. auritus is a gleaning bat (defined as capturing prey crawling or at rest on solid surfaces rather than in flight) (Swift and Racey, 1983; Anderson and Racey, 1993; Swift, 1998) that feeds predominately on Lepidoptera and Diptera (Swift and Racey, 1983; Rydell, 1989; Shiel et al., 1991; Vaughan, 1997). Detailed published information on *P. auritus* nocturnal behaviour is limited to one study in Germany which radio-tracked six females (all non-reproductive or lactating) (Fuhrmann and Seitz, 1992) and one published study in the UK which radio-tracked 16 bats over a total of 65 nights in north-east Scotland (10 female, all non-reproductive or lactating, and 6 males, all mature) (Entwistle et al., 1996). Both of these studies have indicated that this species forages preferentially in broadleaved deciduous woodland which concurs with the foraging preference for *P. auritus* in Southern England (Murphy et al. in prep). The radio-tracking studies in the UK have also demonstrated that female *P. auritus* rarely travel more than 2.5 km from the roost colony (Entwistle et al., 1996; Murphy et al., in prep) and have shown a high night to night consistency in the use of feeding sites (Entwistle et al., 1996). Having regular access to a suitable foraging area within this distance may be necessary for *P. auritus* to successfully raise young and repel intruders from foraging areas thereby reducing competition for resources within them.

This Chapter describes two studies. The first, detailed in Section 5.2 was a pilot study in which radio-tracking techniques were refined and suitable methodology for the analysis of radio-tracking data, in relation to home range use, was evaluated. The subsequent study, detailed in Section 5.3, ascertained nocturnal foraging behaviour, activity patterns and home range estimates for individual female *P. auritus* throughout the active foraging season (May – September), and identified key feeding localities for individuals.

5.2: Pilot Study

5.2.1 – Introduction

The aim of the pilot study was to assess suitable radio-tracking techniques and to review appropriate home range analysis methodology in order to design a subsequent radio-tracking study.

5.2.2 - Methods

5.2.2.1- Study area and capture of bats

Between 6 July and 30 September 2006 a total of six *P. auritus* (five female bats and one male bat) were radio-tracked at Plashett Wood, East Sussex (Grid Reference for centre of site OS TQ 546117 115659) and Ebernoe Common West Sussex (Grid Reference for centre of site OS SU 497745 127192). Bats were caught in either mist nets or harp traps, to which they were attracted using an acoustic lure (Sussex Autobat: Hill & Greenaway, 2005). Capture sites were located inside the wood away from edges and rides and trapping began approximately 60 minutes after sunset. This was done to maximise the chances of catching bats that were foraging rather than travelling to or from their roosts.

5.2.2.2 – Marking bats

Holohil LB-2 (Holohil Systems Ltd.) radio-transmitters were used for tagging the bats. The radio-transmitters weighed approximately 0.5 to 0.6 g including glue which represented approximately 5.9% – 7.1% of the bats weight (based on the mean weight range of the

captured bats). Transmitters were attached with Skin-Bond® (Pfizer Inc) to the area between the shoulder blades from which fur had been clipped. Recapture data indicates that the tags usually fall off the animals after 5 - 12 days. All animals were caught and handled under licence from Natural England. To determine the position of tagged bats during the night the animals were radio-tracked on foot using a Biotrack 'Sika' receiver and a Yagi 3-element antenna on a height-adjustable and portable mast.

5.2.2.3 - Radio-tracking technique and regime

A focal bat's position was determined by taking paired bearings sequentially from various known locations around the foraging area. Each location was 40 - 200m (depending on whether a triangulation could be obtained) from the next nearest location and could be reached by walking in less than one minute. Time, compass bearing, GPS reading and weather were recorded on data sheets in the field. The method of data collection was refined during the course of the study. Animals were radio-tracked from 1.5 hours after sunset (to exclude time spent commuting from their roosts to their foraging sites) for a period of 4 – 7 hours per night in July and August (mean = 4.5hr, SD = 1.08, n = 12) and for a period of 2 – 4 hours per night in September (mean = 3.16hr, SD = 1.32, n = 6). Only active fixes were recorded (to avoid biasing the dataset in favour of roost locations) and bats radio-tracked in September 2006 often returned to their roosts within 2 hours of emergence during adverse weather conditions (windy, heavy rain and low temperatures and less than 8^{o}C).

5.2.2.4 – Data analysis

The location and bearings were used to determine the bat's position using LOAS (version 4.0b, Ecological Software Solutions, 1998 - 2005). The estimated fixes obtained from LOAS

were subsequently imported to Biotas (version 1.03, Ecological Software Solutions, 1998 - 2003) producing a visual representation of the estimated foraging locations. The cumulative home range size was plotted against the number of successive locations for all bats radio-tracked in 2006 in order to determine the point at which the foraging area reached an asymptote, indicating that sufficient data had been gathered from each bat. Estimates of range size for this analysis were based on 100% minimum convex polygons.

5.2.2.5 - Home range analysis

The home range of an individual animal is typically constructed from a set of location points that had been collected over a period of time, identifying the position in space of an individual at many points in time. The concept of home range was described by (Burt, 1943) as 'the area, usually around a home site, over which the animal normally travels in search of food'. However, there is no standard definition of use and interpretations vary (Boulanger and White, 1990; Harris et al., 1990). Harris et al. (1990) argued that the choice of data to include in a home range estimate should depend on the aim of the analysis. If the aim is to detail the entire 24 hour period, then fixes should be collected evenly throughout this period. In these circumstances, many species ranges would, therefore, be centred on a lying up area (i.e. roost area for bats). However, if the aim is to describe the area used whilst foraging, then only active fixes should be considered for inclusion in the home range analysis.

A variety of analytical tools exist to estimate home ranges. These can be divided into two major classes: (i) minimum linkage approaches that describe ranges as polygons with minimised distances between edge locations; and (ii) probabilistic approaches that estimate the density of locations throughout a range (Harris et al., 1990; White and Garrott, 1990;

Kenward, 2001). Current analytical studies on animal home range use tend to employ both methodologies (Nicholls and Racey, 2006; Davison et al., 2009; Jhala et al., 2009). The Minimum Convex Polygon (MCP) (Mohr, 1947) is the oldest and most commonly used method of estimating home ranges and it facilitates comparisons with other studies. The peripheral locations of an animal are joined to each other using a connecting rule. The simplest and most popular connecting rule is simply to connect the outermost points on the scatter of mapped locations such that the sum of linkage distances between edge points is minimised. However, MCPs are very sensitive to outliers and require large data sets for accurate estimations of home range size (Powell, 2000). Furthermore, they give no information about how the animal is using its home range (Harris et al., 1990).

Probabilistic approaches to home ranges estimators have also been developed whereby the density of fixes is estimated throughout the area used by the animal. These approaches are less sensitive to sample size and outliers. Early methods estimated density as ellipses (Calhoun and Casby, 1958) by assuming that locations were distributed normally about one nucleus of activity. Ellipses do not define range shape well but require few locations to reach a maximum estimate and are, therefore, useful for identifying habitat available to animals that cannot be tracked frequently (Kenward et al., 2006).

The harmonic mean model (Dixon and Chapman, 1980) estimates the location density distribution (equivalent to the probability of encountering the animal) at intersections of an estimation matrix. Contours containing a specified percentage of actual locations or estimated location density are then interpolated across the matrix (Dixon and Chapman, 1980). Harmonic mean contours are sensitive to intersection spacing but provide contours least sensitive to outlying locations and are most precise in fitting core locations (Kenward et al.,

2006). This method also has the advantage that the boundary does not have to be elliptical (unlike a normal distribution) and it does not have to be one enclosed area (Harris et al., 1990). Harris et al., (1990) recommend using 80% isopleths, when the distribution of estimated locations are skewed and their number is low, to give a more accurate range representation (fewer than 150 fixes per sample is considered low; Kenward, 1992).

Kernel density estimation (KDE) (Silverman, 1986; Worton, 1987; Worton, 1989) is a non-parametric estimator that describes home ranges by means of hierarchical probabilities for the intensity of habitat utilisation, termed isopleths. The isopleths mainly depend on the probability distribution of single locations on an underlying two dimensional grid. A smoothing factor or bandwidth '*h*' defines the shape and width of this probability distribution. The resulting grid of summed influences represents the probability that an animal would be present at a certain point in its range at any given moment (Worton, 1987). Series of isopleths can be plotted around the smallest area where the cumulative probability reaches a particular value. For example, the 95% isopleth encompasses the area within the probability of finding an animal is 95%. KDE is mathematically robust and produces more consistent results than harmonic mean contouring, but is more sensitive to outlying locations (Kenward et al., 2006).

Taking these considerations into account, range analyses were conducted on location data that excluded commuting and 'inactive' fixes (i.e. periods of hanging up). Two home range estimators were used to calculate total home range for radio-tracked bats once the tracking period for each individual had finished: 100% minimum convex polygons and 80% isopleths harmonic mean. Once data had been collected from the first bat radio-tracked, a comparison of harmonic mean and fixed kernel analysis was carried out on the data points (fixed kernel

was initially chosen over adaptive kernel as data from only one individual existed at that stage). The comparative analysis, detailed in Figure 5.1, revealed that for this individual, kernel analysis better matched the distribution of the fixes. However, the resulting range estimates were very small and almost exclusively centred upon the fixes.

The harmonic mean method resulted in a larger range area which included habitat between fixes. A low number of fixes (40) was obtained for this female throughout the radio-tracking period (mean percentage contact time during radio-tracking was 87%) and, as a consequence, it was likely that kernel analysis would be not be appropriate to represent range use for this female. A study carried out by (Seaman et al., 1999) into the application of kernel methods used computer simulations of 10-200 points / home range to investigate the optimum number of observations that should be obtained for kernel estimates. Seaman *et al*. (1996) recommended that home range studies using kernel estimates should at least acquire a minimum of 30 observations per animal but preferably more than 50. Therefore, it was decided that the 80% HM isopleth would be adopted for this pilot experiment (given the likelihood that the number of fixes for each individual may be low). However, once the data had been collected from all radio-tracked bats in the pilot study, this was re-analysed using Kernel methods to ensure the appropriate home range analysis methodology was employed for the subsequent radio-tracking study detailed in Section 5.3.

5.2.2.6 - Core area analysis

Home ranges usually comprise heterogeneous areas determined by physical and biological factors which explain the disproportionate use of space by animals. Studies on various species show that, for a number of environment-related reasons, certain portions within the

home range are visited more frequently than others (Adams and Davis, 1967; Dixon and Chapman, 1980; Georgii, 1980; Macdonald and Courtenay, 1996). The centre(s) of activity can be defined as the area within the home range in which the most fixes occurred during the radio-tracking period and can give an indication of which part(s) of the range the bats used more intensively. Areas of more intensive use have been termed as the 'core area of the home range' of the animal and may be related to the greater availability of food resources and refuges (Samuel et al., 1985; Thompson et al., 2007).

Probabilistic home ranges (such as those derived from HM or KDE) allow identification of core areas by plotting the percentage of maximum probability of use (i.e. 10% isopleth, 20% isopleth etc.) on the x axis against the percentage of home range contained within the isopleth on the y axis (Harris et al., 1990; Powell, 2000). The resulting relationship shows: i) a descending straight line with a slope of -1 if space use is random; ii) a curve sagging below the line if space use is clumped; or iii) a curve above the line if space use is uniform. If a clumped relationship is shown, the core area can be identified at the point on the graph where the distance between the curve and the straight line is the greatest (Harris et al., 1990; Powell, 2000). The corresponding isopleth can be read off the graph. This analysis, termed 'utilisation plots' (as described by Powell, 2000), was used to assess whether bats had core areas that they use more intensively than other parts of their ranges. Core areas were identified by constructing utilisation plots, using figures derived from 80% Harmonic Mean analysis. This revealed that animals invariably showed clumping in their space use (Figure 5.2) with maximum clumping occurring between the 30% and 50% (Table 5.1). The median value of 40 % was adopted and applied to all analyses as a comparable estimate of core area.

5.2.3 - Results

5.2.3.1 - Ranging Area

Radio transmitters were attached to a total of six adult female bats and one adult male bat but data was obtained from only five of the six female bats (as one transmitter failed on *Plecotus auritus* before any data could be collected). Radio-tracking data from the first night after capture was not used in the analysis as a study on *Myotis bechsteinii* by (Fitzsimons et al., 2002) found that some tagged bats spent the night they were captured on their own, away from the main roost, which may have been as a direct result of being tagged. However, on the first night after capture, an approximation of the key foraging areas for each bat was determined which made subsequent nights radio-tracking easier. The six bats (5 female and 1 male) were radio-tracked for up to six hours per night for a total of 76 hours resulting in 311 estimated locations that were used for analysis. Data for two bats (one male, bleb 5, and one female, bleb 7) did not reach asymptote, as shown in Figure 5.3.

The 100% MCP and 80% HM home range estimators for all five bats radio-tracked at Plashett Wood were defined as shown in Figure 5.4 and Figure 5.5 respectively. Both the 100% MCP and 80% HM show that there was considerable overlap in home range amongst the radio-tracked individuals (however, as the individuals were not simultaneously radio-tracked, it is not possible to be conclusive about concurrently shared foraging areas). The 40% HM core foraging area for all five bats radio-tracked at Plashett Wood was defined, as shown in Figure 5.6, and there was overlap for two bats observed (however, again, this is not conclusive). The home range analysis for the bat radio-tracked at Ebernoe Common was defined as shown in Figure 5.7. This female had two clear foraging locations, one in Ebernoe

Common adjacent to her roost and a secondary foraging area in The Hoe approximately 1 km from her primary foraging area. The size of foraging area for each bat is detailed in Table 5.2. One bat (Bleb 7), had a home range (80% HM) which was 5 times greater in size compared to the mean of the other five bats and a core foraging area which was 4 times greater in size compared to the mean of the other bats. This bat was radio-tracked in poor weather conditions (very windy and high rainfall) and did not reach asymptote with respect to her foraging range size. She foraged predominately over hedgerows en route from her roost to Plashett Wood. Figure 5.8 shows the mean range size of all bats including and excluding this female.

5.2.3.2 - Ranging Analysis

At the end of the pilot experiment a re-analysis of all home range methodologies revealed that the use of harmonic mean methodologies produced large home range estimates and included substantial areas of habitat which were not visited by individuals. When the number of fixes exceeded 40, fixed kernel analysis more closely matched the distribution of the fixes for *P. auritus*, as shown by Bleb 4, Figure 5.9. The use of harmonic mean analysis for Bleb 7 (the female which did not reach the asymptote) resulted in a range that was vastly greater in size compared to the other females. This female foraged along hedgerows and a copse, as detailed by the estimates of foraging locations in Figure 5.10, and the resulting comparative analysis of HM and KDE, shown in Figure 5.11, revealed that KDE was more likely to be a more accurate representation of range use. However, the use of KDE gave significantly smaller estimates of home range size for both peripheral (Wilcoxon n=6, $Z = 2.201$, $p = .028$) and core areas (Wilcoxon n=6, $Z = 2.201$, $p = 0.28$) compared to harmonic mean estimators, as detailed in Figure 5.12.

5.2.4 - Discussion

5.2.4.1 - Range use

In this pilot study, range use of *P. auritus* was characterised by small home ranges and high site-fidelity. Small home range size is consistent with previous findings from radio-tracking studies on *P. auritus* by (Fuhrmann and Seitz, 1992; Entwistle et al., 1996; Murphy et al. (in prep.). The radio-tracking in this study was carried out over a mean radio-tracking period of 4.10 hours per night per individual (SD = 1.32; n = 6). Although asymptote was reached for four of the individuals, it is possible that a larger area would have been obtained for each bat if radio-tracking had taken place throughout the entire night. During the three nights that the six bats were radio-tracked, all returned to the same feeding areas on consecutive nights and each remained loyal to a particular area. Given the high consistency of range use it is considered that the identification of core and peripheral foraging areas is meaningful for *P. auritus* during a short term of radio-tracking study.

5.2.4.2 - Effect of transmitters on bat behaviour

A study by (Gaisler et al., 1980 - 1981) found that their survey methods, which included the capture and handling of bats, were negatively influencing the bat population they were studying. However, a study by (Hickey, 1992) compared the foraging success of hoary bats, *Lasiurus cinereus*, with and without radio-transmitters and found that there was no significant difference. Ringed and radio-tracked bats have been re-found year after year (Kurta and Murray, 2002; Neubaum et al., 2005; Greenaway, 2008) and the general hardiness and longevity of these animals may indicate that this is not a problem. During the study, although

fixes from the first night of radio-tracking were omitted from the analysis, all bats returned to the area in which they had been captured and utilised the same foraging areas on subsequent nights which suggests that capture, handling and tagging did not unduly affect their behaviour. Nonetheless, it cannot be ruled out that stress, due to handling and marking, may have altered the individual's behaviour.

5.2.4.3 - Radio-tracking technique

Normally a radio-tracking study requires a triangulation technique, with two or more direction bearings obtained from receivers at known locations (White and Garrott, 1990). Due to a lack of manpower available during the pilot study, the only available methodology was to take single bearings using one receiver at various locations around the animal's foraging range. Even though the minimum time possible was left between taking bearings, so that the bats movement in this time was also there was the potential for some of the estimated locations to be inaccurate due to the movement of the bat in the time between each of the bearings being recorded. Sometimes bats flew rapidly from one location to the next and, therefore, it was considered that the single bearings approach may be inappropriate for this species and triangulation would yield more accurate results.

5.2.4.4 - Home range estimators

The implementation of any home range estimator will have an important effect on the results of estimated home range use in any radio-tracking study. The use of the harmonic mean methodology for the analysis of home range demonstrated that when individuals, such as Bleb 7, foraged along hedgerows, the home range area estimated appeared greatly over-

inflated in size compared to the distribution of the actual estimates of locations. A re-analysis of the estimates of locations, using Kernel Density Estimators (KDE), showed that the resulting areas better matched the distribution of the fixes. The Kernel method produces a nonparametric estimation of a distribution range based on a random sample of point observations. Each kernel is a density, so the resulting distribution range estimate is a true probability density function (Worton, 1989). The selection of a fixed bandwidth (h), however, may have important consequences for range estimates (Gitzen et al., 2006). A large bandwidth can lead to oversmoothing resulting in a large polygon encapsulating all fixes, with no internal definition, whereas a small bandwidth can lead to undersmoothing resulting in individual rings around each fix (Kenward, 2001). Undersmoothing is likely to have occurred in the analysis of KDE, particularly for Bleb 2 (see Figure 5.1) which had a low number of fixes.

5.2.5 - Conclusions

P. auritus used the same feeding areas on consecutive nights and each remained loyal to particular foraging areas. All night radio-tracking from dusk to dawn (as opposed to a fix time schedule) would increase the likelihood of incorporating all feeding localities during the foraging period. Radio-tracking from two positions, with two observers (communicating via two way radios) would allow for simultaneous and more accurate bearings to be collected.

Analysis of home range methodologies revealed that kernel density estimators better matched the distribution of *P. auritus* fixes compared to harmonic mean methodologies. However, the selection of an appropriate bandwidth (h) may have important implications for final range size estimates.

5.3: Ranging behaviour of female *P. auritus*

5.3.1 – Introduction

The pilot study, described in Section 5.2, established the appropriate methodological techniques and subsequent analysis. This was used to inform a detailed radio-tracking study of female *P. auritus* described in this section.

5.3.2 - Methods

5.3.2.1 - Study Area

The study area consisted of seven woods at four sites, three in West Sussex and one in East Sussex (Figure 5.13). All sites were in broadleaved deciduous woodland dominated by oak. NVC analysis identified all the woods as being W10 *Quercus robur, Pteridium aquilinum, Rubus fruticosus* woodlands. This is the most common woodland type on lowland sites in south east England (Hall, 2004). The sites were chosen because previous work had indicated the presence of reproductive *P. auritus* females (Murphy *et al.* in press; unpublished data). One wood was owned and managed by the Sussex Wildlife Trust and six were privately owned. The woods were managed for a variety of purposes including forestry, pheasant shooting and wildlife conservation. All of the woods had good cover in the canopy layer and a well developed understorey of woody shrubs (see Murphy *et al.* in prep for further detailed of woodland structure and *P. auritus* use of woodlands).

5.3.2.2 - Capture and selection of study animals

Between July 2007 and September 2008 a total of 24 female *P. auritus* bats were captured at the study sites and fitted with radio-transmitters. Bats were caught in either mist nests or harp traps, attracted by the use of an acoustic lure (Sussex Autobat: Hill & Greenaway, 2005) that produced synthesised social calls of *P. auritus* (as detailed in Chapter 4). Capture sites were located in the woodland interior, away from edges and rides, and trapping began approximately 60 minutes after sunset. These measures were taken to maximise the chances of catching bats that were foraging rather than ones commuting to or from their roosts. Trapping for bats was suspended in both years from 20th June – 10th July in order to avoid causing distress to heavily pregnant females or separating adult females from their young pups.

5.3.2.3 - Radio-transmitters and tracking methods

Each female was fitted with a Holohil LB-2 (Holohil Systems Ltd.) radio-transmitter. Fur was clipped from the area between the bat's shoulder blades and the transmitter was attached using Skin-Bond ® (Smith & Newphew Inc) surgical adhesive. The tags weighed 0.5 – 0.6g including glue, which was equivalent to 7% of body mass (average across all females; range 5.9% and 7.2%) and was always less than 10%, as recommended by Wilkinson & Bradbury (Wilkinson and Bradbury, 1988). Radio-tracking records indicate that tags usually fall off the animals after 5 - 12 days (unpublished records).

The animals were radio-tracked on foot, by two surveyors, using Sika radio-receivers (Biotrack Ltd) and Yagi 3-element antennas mounted on portable masts with an adjustable height up to 4.2m, to increase the detection of the signal due to the unevenness of the terrain.

The position of the focal bat(s) was communicated by the surveyors using two-way radios (Motorola AU1200). The bat's position was determined by synchronised triangulation from various known locations (determined by GPS Garmin eTrex Legend HCX) around the bat's foraging area. Time, compass bearing, location, weather conditions and notes were recorded on data sheets in the field. These details were recorded approximately every 5 minutes when in contact with the focal animal(s) during the tracking period, with the aim of providing estimates of location that could be plotted onto mapping software and analysed. The surveyors took 15 minute recording breaks approximately every two hours but tried to synchronise this with periods of the focal bat(s) inactivity. In 2007 and 2008 a total of 10 pairs of female *P.auritus* (termed dyads) were radio-tracked synchronously (see Chapter 7 for detailed analysis for range overlap and interaction analysis). Synchronous radio-tracking involved taking a set of bearings from one individual from the dyad followed immediately by a set of bearings from the other member of the dyad.

The effect of autocorrelation (i.e. correlation between the locations of successive 'fixes') on home range estimates has been the subject of debate amongst researchers. Several authors have argued that frequent monitoring of an individual's location via radio-tracking or direct observation severely jeopardises the validity of the independence assumption (Dunn and Gipson, 1977), and that frequent successive observations will tend to be positively correlated which results in a underestimate of the true size of the home range (Swihart and Slade, 1985a; Swihart and Slade, 1985b; Harris et al., 1990).

However, more recently, it has been argued that autocorrelated data is needed to adequately represent the non-random movement that many animals exhibit (Rooney et al., 1998; De Solla et al., 1999; Otis and White, 1999). Using simulated data, de Solla *et al.* (1999)

demonstrated that the accuracy and precision of home range estimates improved at shorter time intervals despite the increase in autocorrelation among the observations. They concluded that the ideal data collection regime would be one where data was collected as frequently as possible, at evenly spaced intervals throughout the study period. Therefore, in this study, the aim of the tracking was to obtain as much information on the bat's use of home range during the short period of radio-tracking (due to constraints on the lifespan of the radio-transmitters used which, in turn, is determined by the body mass of *Plecotus auritus*). Although this meant that data points for radio-tracked individuals were temporally correlated, it was the only practical method available given the short duration of the study. The potential bias of collecting data on unrepresentative nights (nights where low temperatures ($< 8^{o}$ C) and/or excessive rainfall (more than 30 minutes of continuous rain), resulting in extended periods of inactivity) was minimised by excluding fixes near roost locations and during periods of inactivity (i.e. where the bat was 'hanging up').

There are biases to radio-tracking studies that need to be accounted for when analysing species foraging ranges. When two bearings are used to triangulate a tagged animal's position, errors can arise in several ways (Heezen & Tester, 1967; cited in Kenward 2001). Systematic errors can occur from misalignment of the compass, or readings taken when the compass is too close to a large metal object (such as the receiver) (Kenward, 2001). Some bearings may also not triangulate accurately which may be a consequence of the bat moving rapidly from one area to another in the time between each bearing being recorded (Schmutz and White, 1990). In this study this source of error was avoided by two field workers taking simultaneous bearings. Nevertheless, approximately 2% of the overall bearings did not triangulate, which may have been a result of human error.

Data from each night of radio-tracking was added to a cumulative database and for each individual this was used to estimate the bat's ranging area (see below). Individual females were radio-tracked for as many nights as was required for the cumulative ranging areas to reach asymptote, as recommended by Kenward (2001) and Laver and Kenward (2008).

5.3.2.4 - Home range analysis

Pairs of compass bearings, and the locations they were taken from, were used to estimate the bat's position (fix) by triangulation with the software package LOAS (version 2.12, Ecological Software Solutions, 1998 – 2003). The fixes obtained from LOAS were imported into Ranges 7 (version 1.8, Anatrack Ltd, 2006) and ArcGIS 9 (version 9.2, ESRI 2006) which were used to produce visual representations of the estimated ranging areas.

Two range estimators were used to calculate total range estimates for each individual: 100% MCP and 95% KDE (see Section 5.2). Kernel analysis was used to produce a probability density surface over fixes (Silverman, 1986). The influence of points is estimated by a bivariate normal estimator and the distance of which a point has an influence is controlled by the bandwidth (h). However, the selection of bandwidth can have a substantial effect on the size of home range estimates (Gitzen et al., 2006). The reference bandwidth (href), which is a function of the variance in the fix co-ordinates, tends to oversmooth and hence overestimate range size if an animal has more than one area of high activity (Worton, 1989). To overcome this problem, Worton (1989) suggested the use of either least-squares cross validation (LSCV) to select an appropriate multiplier of href or, alternatively, allowing the value of h to vary throughout the range (known as adaptive kernel analysis). Therefore, individual home ranges were estimated as adaptive kernels with the smoothing factor *h* estimated from the

data and optimised via least square cross validation, LSCV (h_{cv}; Worton, 1989). The females' home ranges were calculated with a mean h_{cv} to keep home ranges comparable among individuals (Kenward, 2001). The data sets for each bat used for home range analysis contained more than 30 fixes (mean number of fixes = 161.04, SD = 30.57, n = 24) as recommended by (Seaman et al., 1999). (Silverman, 1986) reported a bias in LSCV estimators of clumped data points. However, the exclusion of duplicate fixes at roost and 'hanging up' (i.e. night roosting locations) sites eliminates the risk of overestimation of habitat use around roosts due to, for example, swarming behaviour.

5.3.2.5 - Core areas

Core areas can be a useful concept when describing patterns of behaviour or identifying particularly resources (Harris et al., 1990; Powell, 2000). All bats showed clumping in their use of space (see Figure 5.14) with maxima occurring between the 35% and 65% isopleths (Table 5.3). The 50% isopleth (median value) was adopted as an indicator of core area use. The use of 50% KDE for *P. auritus* core use in this study, would also make it comparable with other radio-tracking studies on woodland bats, for example, Barbastelle *Barbastella barbastellus* (Hillen et al., 2009), (Kerth and Melber, 2009) Bechstein's *Myotis bechsteinii* (Dietz and Pir, 2009; Kerth and Melber, 2009) and lesser horseshoe *Rhinolophus hipposideros* (Bontadina et al., 2002). The 50% isopleth was, therefore, adopted as an indicator of core areas.

5.3.2.6 - Statistical analysis

The emergence time of radio-tagged females in relation to sunset and civil twilight was examined using regression analysis. For the analysis of radio-tracking data, separate analysis of variance (ANOVASs) was carried out for the mean MCP, mean peripheral foraging area and mean core foraging area. Reproductive status (pregnant, lactating, post-lactating) was entered as a factor in order to test for any association between reproductive condition and range size. Data was transformed so that the residuals were normally distributed whenever possible.

5.3.3 - Results

Radio transmitters were attached to a total of 24 female bats and data was obtained from all 24 radio-tracked individuals. Details of female *P. auritus* radio-tracked over the two years are shown in the Appendix Table A.6.1. The 24 bats were radio-tracked over 56 nights from emergence to re-entry, resulting in a total of 3865 fixes that were used for analysis. On 36 of these nights, a total of 20 of the 24 individuals were radio-tracked simultaneously in pairs (see Chapter 7).

5.3.3.1 - Nocturnal activity of radio-tagged females

Broadly speaking, the nocturnal behaviour of *P. auritus* could be defined as one of three activities.

i) Foraging: Bats spent time flying in discrete locations (termed foraging areas) and during this activity it was assumed that the bat was seeking or consuming prey. Within these areas, bats were occasionally observed flying slowly in and out of trees with a dipping flight path, as described by (Entwistle et al., 1996).

ii) Resting: Females were assumed to be inactive or 'hanging up' when the signal was steady without amplitude modulations which typifies a moving animal (Burger et al., 1991; Krull et al., 1991; Kenward, 2001).

iii) Commuting: This typically involved fast directional flight to and from the roost site.

The 24 bats radio-tracked in the study utilised a combination of tree and house roosts (see Murphy *et al.* in prep for detailed analysis of differential characteristics between roosting in tree and house roosts). The median emergence time of radio-tagged bats occurred 38 minutes after sunset (mean = 42.1 min, SD = 27.5, n = 81) and 6 minutes before the end of civil twilight (mean = 7.4 min, SD = 26.65, n = 81). The times of emergence for each female, on each night, in comparison to sunset, are shown in Figure 5.15 (a) and in comparison to civil twilight are shown in Figure 5.15 (b). The time of emergence was highly correlated with both the time of sunset (Spearman rank correlation, $r_s = 0.878$, n = 81, $p < 0.01$) and with the time of civil twilight (Spearman rank correlation $r_s = 0.875$, n = 81, $p < 0.01$). However, two individuals, one adult and one juvenile (Bleb 25 and 26) simultaneously radio-tracked, utilised a tree roost together within their foraging range, and emerged later than other tagged females (up to 2.5 hours after sunset) during the radio-tracking period (which may have been as a consequence of adverse weather conditions during that period).

The median time for final re-entry to roost was 36 minutes before sunrise (mean = 38 min, SD = 14.12, n = 52) and 1 minute after the beginning of civil twilight (mean = 1 min, SD 10.62, n = 52). The times of final re-entry to roost for each female, on each night, in comparison to sunrise, are shown in Figure 5.16 (a) and in comparison to the civil twilight are shown in Figure 5.16 (b). The time of returning to the roost was also highly correlated with both the time of sunrise (Spearman rank correlation $r_s = 0.940$, n = 52, p <0.01) and with the time of civil twilight (Spearman rank correlation $r_s = 0.932$, n = 52, p <0.01). The times of emergence varied between roost sites but there was so significant difference between mean emergence times of females roosting in trees compared to females roosting in buildings (Kruskal-Wallis ANOVA H = .142, n = 24, p < 0.706), and the mean time of emergence for each roosting location was not significantly correlated with the distance to the closest woodland (Spearman rank correlation $r_s = 0.038$, n = 24, p < 0.858).

The nocturnal activity pattern of females was characterised by bouts of flight combined with short periods of inactivity, as described by Entwistle et al. (1996). The periods of inactivity, ranging from one minute to 85 minutes (median = 16 min), were predominately spent in trees (seventy two occasions), including trees used as day roosts and, less often (four occasions), in buildings used as day roosts. Each of the buildings used was situated within or at the edge of broadleaved woodland. Both lactating and non-lactating females re-entered the maternity roost during the night, but lactating females (n = 6) re-entered more frequently compared to pregnant, non-reproductive and juvenile females (n = 18) (Kruskal-Wallis ANOVA H = 13.66, n = 24, p < 0.001). Excluding the lactating females (as periods of inactivity could not be separated from time feeding offspring), the mean hours of rainfall recorded during the radio-tracking period was significantly correlated with mean time spent inactive (Spearman rank

correlation r_s =0.860, n = 18, p < 0.01) as shown in Figure 5.17 (a) and negatively correlated (although weaker) with mean minimum temperature recorded during the radio-tracking period (Spearman rank correlation r_s = -0.683, n = 18, p < 0.01) as shown in Figure 5.17 (b). A partial correlation between inactivity and rainfall (controlling for temperature) did not find a significant correlation (r = -0.389 n = 18 p = 0.123). However, a partial correlation between inactivity and temperature (controlling for rainfall) did find a significant correlation (r = 0.693 n = 18 p = 0.002), which suggests that temperature may be a more important determinant of inactivity compared to rainfall.

5.3.3.2 - Asymptotes of range size

The point at which the asymptote in the cumulative size of the bat's foraging range was reached was used to indicate if sufficient data had been gathered from each bat to provide an estimate of the extent of home range use. Asymptotes of home-range size were achieved for 22 of the 24 females radio-tracked. Blebs 31 and 32, radio-tracked in September 2008, did not reach the asymptote, but it was decided to terminate radio-tracking as adverse weather conditions during that period may have resulted in the tags failing. Range size plot of all females radio-tracked in 2007 are shown in Figure 5.18 and reveals that range size begins to level off at about 60 fixes and reaches a more stable estimate after ca. 115 fixes.

5.3.3.3 - Foraging characteristics

All individuals foraged within broadleaved woodland and some individuals also utilised mature hedgerows. For all females the majority of foraging fixes were recorded in woodland (including copses) (see Murphy *et al.* in prep for detailed analysis relating to habitat use).

Hedgerows were also utilised by females and there was evidence of seasonal difference in use (see Murphy *et al.* in prep). Most fixes in the other habitat types were associated with trees. For example, gardens all abutted woodland and had trees in them, and bats recorded over water were generally within a few metres of the tree-lined bank of a stream or the edge of a woodland pond or lake. Figure 5.19 shows the spatial distributions for four females, which were radio-tracked at Plashett Wood in 2007 (overlaid on an OS map of the area). The 95% KDE and 50 % KDE demonstrates that females predominately focus their foraging in broadleaved woodland.

5.3.3.4 - Home range size

Minimum Convex Polygon (100%) home ranges of individual females varied between 2.7 ha and 19.42 ha (mean = 7.42 ha, n = 24, SD = 3.47; Table 5.4). Kernel density estimates of home ranges of individual females varied between 1.64 ha and 10.16 ha (mean = 4.54ha, n = 24, SD = 1.99; Table 5.4). Reproductive status (pregnant, lactating, post-lactating) had no significant effect on range size regardless of which type of range estimator was used (MCP: $F=0.294$, d.f. = 23, $p = 0.651$; 95% KDE: $F = .165$, d.f. = 23, $p = 0.514$). Figure 5.20 shows the spatial distribution of the 100% MCP and Figure 5.21 shows the spatial distribution of the 95% KDE for eight female radio-tracked in Plashett Wood in 2007. There was overlap between the ranges of different individuals radio-tracked within the same year (including those radio-tracked simultaneously, analysed separately in Chapter 7).

5.3.3.5 - Core area use

Core areas, as defined by the 50% KDE, were areas individual *P. auritus* females used on successive nights and these were characterised by between one and seven locations within their home range (median = 2). These core areas of individual females varied between 0.69 ha and 5.39 ha (mean = 2.38 ha, n = 24, SD = 1.08). In general, females tended to return to these areas to forage on a regular basis each night. Reproductive status (pregnant, lactating, post-lactating) had no significant effect on core area (50% KDE: F = .172, d.f. = 23, p = 0.492). Figure 5.22 shows the spatial distribution of the 50% KDE for each female radio-tracked in Plashett Wood in 2007. There was, also, overlap in core area use between different individuals radio-tracked within the same year (including those radio-tracked simultaneously, analysed separately in Chapter 7). The mean size of all home range estimators (100%MCP, 95%KDE and 50% KDE) for all females radio-tracked in 2007 and 2008 is shown in Figure 5.23.

5.3.4 - Discussion

5.3.4.1 - Nocturnal activity of radio-tagged females

The median emergence time of *P. auritus* in this study was 38 minutes after sunset at 51^0N, similar to the median emergence time of *P. auritus* at the same latitude found by (Battersby, 1999) (36.4 minutes after sunset) and by (Howard, 1995) (40 minutes after sunset) and followed the pattern of other studies whereby median bat emergence was later with increasing latitude: 46^0N, 15 minutes after sunset for a study in France, (Barataud, 1990);

50^0N, 26 minutes after sunset for a study in Germany (Fuhrmann and Seitz, 1992); and at 57^0N, 55 minutes after sunset for a study in Scotland (Entwistle et al., 1996).

The emergence time for bats generally tends to be highly synchronised with the time of sunset (Swift, 1980; Catto et al., 1995; Duverge et al., 2000; Petrzelkova et al., 2006; Reichard et al., 2009) and is likely to be related to the use of light levels as a cue for emergence (Erkert, 1978; Erkert, 1982; McAney and Fairley, 1988; Isaac and Marimuthu, 1993). The evening emergence time appears to be a function of dietary specialisations and foraging strategy and is probably, also, affected by the ability to avoid predation (Jones and Rydell, 1994; Speakman et al., 2000; Reichard et al., 2009).

The median time of roost return of females in this study was 36 minutes before sunrise (1 minute after the beginning of civil twilight). This contrasts with studies carried out in Germany (50^0N) by (Fuhrmann and Seitz, 1992) where *P. auritus* (n = 6) on average returned 186 minutes before sunrise, and also in Scotland (57^0N) by Entwistle et al., (1996) where *P. auritus* (n = 16) returned on average 57 minutes before sunrise. In this study, all the roosts were located either within trees in the woodland or buildings in or at the edge of woodland, connected directly to the woodland by hedgerows and copses. Bats roosting in woodland are less vulnerable to predators and may take advantage of this by prolonging foraging (Russo et al., 2007).

The females roosting outside of the woodland in which they were caught predominately used hedgerows and tree lines for commuting to and from their roosts and foraging sites. The use

of linear features for commuting by bats has been demonstrated for a number of different species including *P. auritus* (Entwistle et al., 1996), *Pipistrellus pipistrellus* and *P. pygmaeus* (Downs and Racey, 2006) and *Rhinolophus hipposideros* (Stone et al., 2009). The use of covered flyways by *P. auritus* may help in avoiding avian predators such as tawny and barn owl (Speakman, 1991).

In Southern England, females flew throughout the night with no substantial period of night roosting, similar to *P. auritus* in Scotland (Entwistle et al., 1996) and other gleaning bats such as *Myotis bechsteinii* (Fitzsimons et al., 2002; Dietz and Pir, 2009), *Myotis evotis* (Chruszcz and Barclay, 2003) and *Myotis myotis* (Rudolph et al., 2009). In contrast to the study carried out in Scotland (Entwistle et al., 1996), both lactating and non-breeding females returned to the roost during the night. However, the fact that in this study female *P. auritus* roosted in trees, often within their foraging range, indicates that the non-lactating females that returned to their roost may have been resting or avoiding adverse weather conditions. Inactivity throughout the night was significantly correlated with temperature and rainfall but when these effects were controlled for, inactivity was significantly was correlated with temperature only. This is in contrast to foraging behaviour in Scotland where inactivity was significantly correlated with rainfall but not temperature (Entwistle et al., 1996). However, the study in Scotland had a small sample size for individuals night roosting on cold nights (n = 7) and it is likely that both temperature and rainfall will have effects on bat activity in different environments and/or habitats (Parsons et al., 2003).

5.3.4.2 - Feeding site fidelity

The knowledge of patches of habitat where high prey availability can be predicted may be a key benefit of remaining loyal to a foraging range, as demonstrated by other animals such as birds (Greenwood and Harvey, 1976; Irons, 1998; Raymond et al., 2010), whales (Valenzuela et al., 2009) and sea lions (Chilvers, 2008). During the time that the 24 female *P. auritus* were radio-tracked, each remained loyal to a particular foraging area. High consistency from night to night in the use of foraging areas has also been shown for bats such as *Macrophyllum macrophyllum* (Meyer et al., 2005) and *Barbastella barbastellus* (Hillen et al., 2009). Other gleaning bats, such as *Myotis blythii* and *M. myotis* (Arlettaz, 1999), *M. emarginatus* (Krull et al., 1991) and *Plecotus austriacus* (Fluckiger and Beck, 1995) have also been shown to use similar sites on successive nights. Studies on *Myotis bechsteinii* (Kerth et al., 2001) and *Myotis myotis* (Rudolph et al., 2009) and *Barbastella barbastellus* (Hillen et al., 2009) have shown that foraging areas for some individuals remained constant over consecutive years.

This study was only able to ascertain loyalty over a period of a few days and it may be that range-use changes over longer periods, expanding or contracting or even shifting foraging areas completely at certain times of the year. There was evidence of seasonal changes in foraging habitat for *P. auritus* in a study by Murphy et al. (in prep), wherein data from 38 females found that hedgerows were the most frequently used habitats after woodland, although their use varied seasonally, with little use before mid July but increasing significantly in August and September. Seasonal changes in foraging behaviour have also been demonstrated for other bat species such as *Nyctalus leisleri* (Shiel et al., 1999) *Nyctalus noctula* (Mackie and Racey, 2007) and *Rhinolophus euryale* (Goiti et al., 2008).

5.3.4.3 - Home range use

The radio-tracking of bats has limitations, such as the short lifespan of the tags, in that the data does not necessarily reveal a full "home range". However, for the purposes of this discussion the focus will be on home range-use at the time of year when the females were radio-tracked. Further limitations to the accuracy of range representation arise due to the accuracy of the bearings, as this is dependent upon the proximity of the receiver to the transmitter which can affect the accuracy of the calculated fixes. Finally, it cannot be ruled out that, as a consequence of tagging, the bat is distressed. However, the females that were caught and tagged in their foraging area remained foraging in that area on subsequent nights, which would suggest that their behaviour was not unduly affected.

There have been no published studies on estimates of home range size for *P. auritus* to date (although see Murphy et al. in prep for details of 38 females which includes data from the 24 females included in this study). Previous radio-tracking studies on *P. auritus* having focused on distance travelled from roosts and habitat use analysis (Fuhrmann and Seitz, 1992; Entwistle et al., 1996). The mean estimated home range for *P. auritus* (100% MCP 7.4ha) is smaller than the mean home range estimates for other UK bat species such, as *Barbastella barbastellus* in Sussex, (Ebernoe Common Colony mean 100% MCP 779ha; (Greenaway, 2008); *Pipistrellus pipistellus* 100% MCP 168ha (Nicholls and Racey, 2006); 100% MCP 157ha (Davidson-Watts and Jones, 2006) *Pipistrellus pygmaeus* 100% MCP 30ha (Nicholls and Racey, 2006); 100% MCP 146ha (Davidson-Watts and Jones, 2006); *Nyctalus noctula* MCP 100% 820ha (Mackie and Racey, 2007), but is comparable with the range size of sympatric woodland gleaning species, such as *Myotis bechsteinii* 95% MCP 1.21ha

(Fitzsimons et al., 2002) and 100% MCP (including commuting routes and roosting localities) 11.2ha (Schofield and Morris, 1999).

5.3.4.4 - Core foraging areas

In this study, females did not utilise all of their home range with equal intensity and returned to specific areas, termed core foraging areas, within their home range more frequently than others. Presumably, these core feeding areas were associated with increased feeding success. A study by Thompson et al., (2007) on the core feeding areas within the home range of East African chimpanzees *Pan troglodytes schweinfurthii* found that the quality of core areas is associated with variance in reproductive success among female East African chimpanzees, whereas females in neighbourhoods containing more preferred food had elevated ovarian hormone production, shorter birth intervals and higher infant survivorship. Vegetation analysis on the composition of *P. auritus* foraging areas (Murphy *et al* in prep.) showed that there was significantly more understorey in terms of cover and, also, species diversity in core foraging areas compared to peripheral foraging areas. Core foraging areas were often characterised by understorey species, such as *Crataegus monogyna* and *Alnus glutinosa*, that support a much greater diversity of insects than species such as *Carpinus betula* (Kennedy and Southwood, 1984) or the non-native *Rhododendron ponticum* (Cross, 1975), which were often the characteristic species present in peripheral areas. As female *P. auritus* rarely travel further than 2km from the colony roost (Entwistle et al., 1996), regular access to key core foraging areas within this distance may be necessary to successfully raise young.

5.4 – Summary

- Individual females clearly had specific ranging areas that they returned to on consecutive nights, although the extent to which these are fixed over longer periods is not known.
- These ranges were small in size in comparison to the size of published home range size of other bat species. Within the home ranges of females were core areas, which females returned to more frequently compared to other areas within their range.

If female responses to synthesised social calls represent defence of resources, then it would be expected that there are differences in response in relation to where the stimulus is played within their home range.

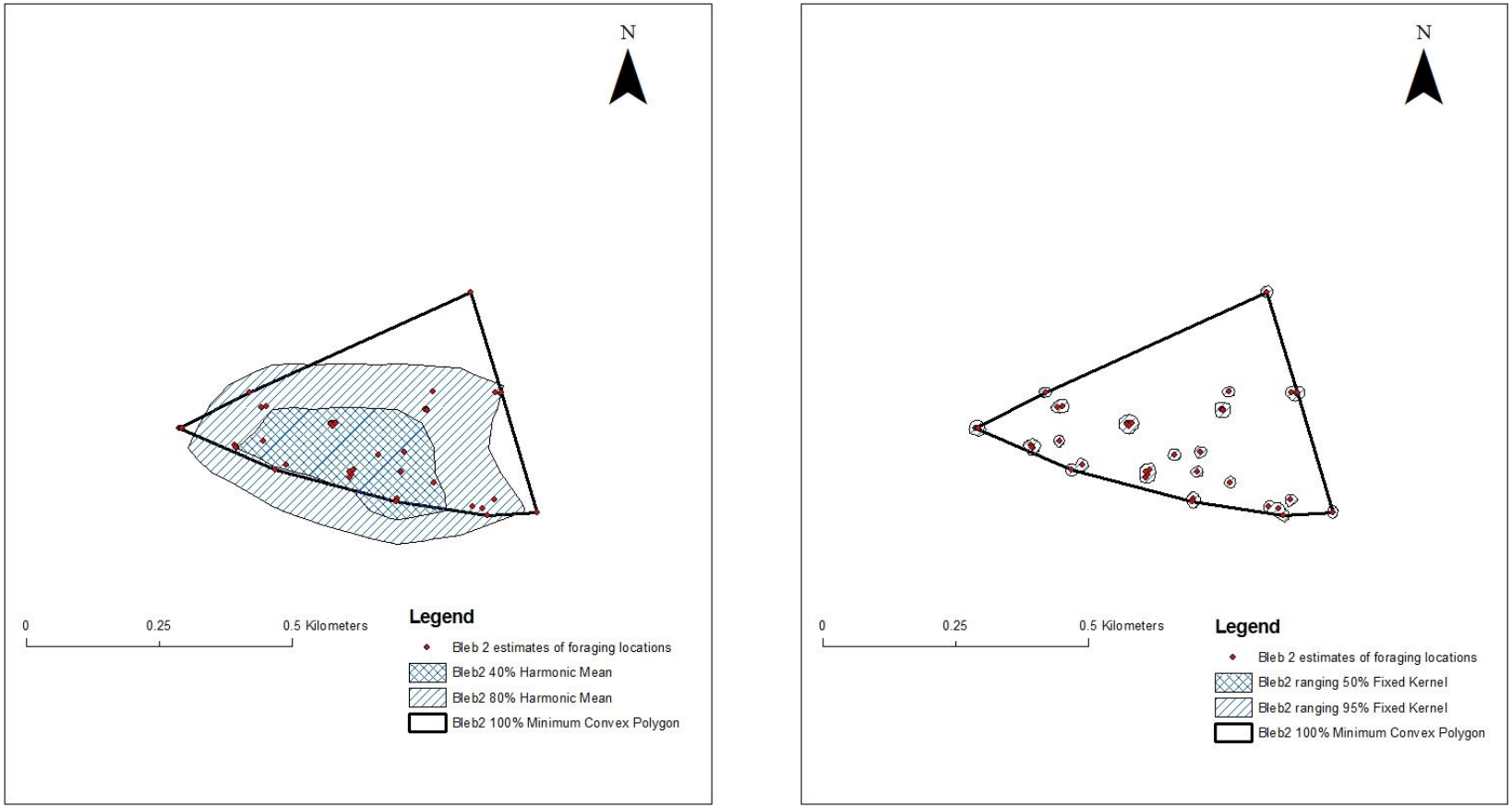


Figure 5.1: Comparative analysis of home range use for bleb 2 using harmonic mean methodology (left) and Fixed Kernel Estimates (right).

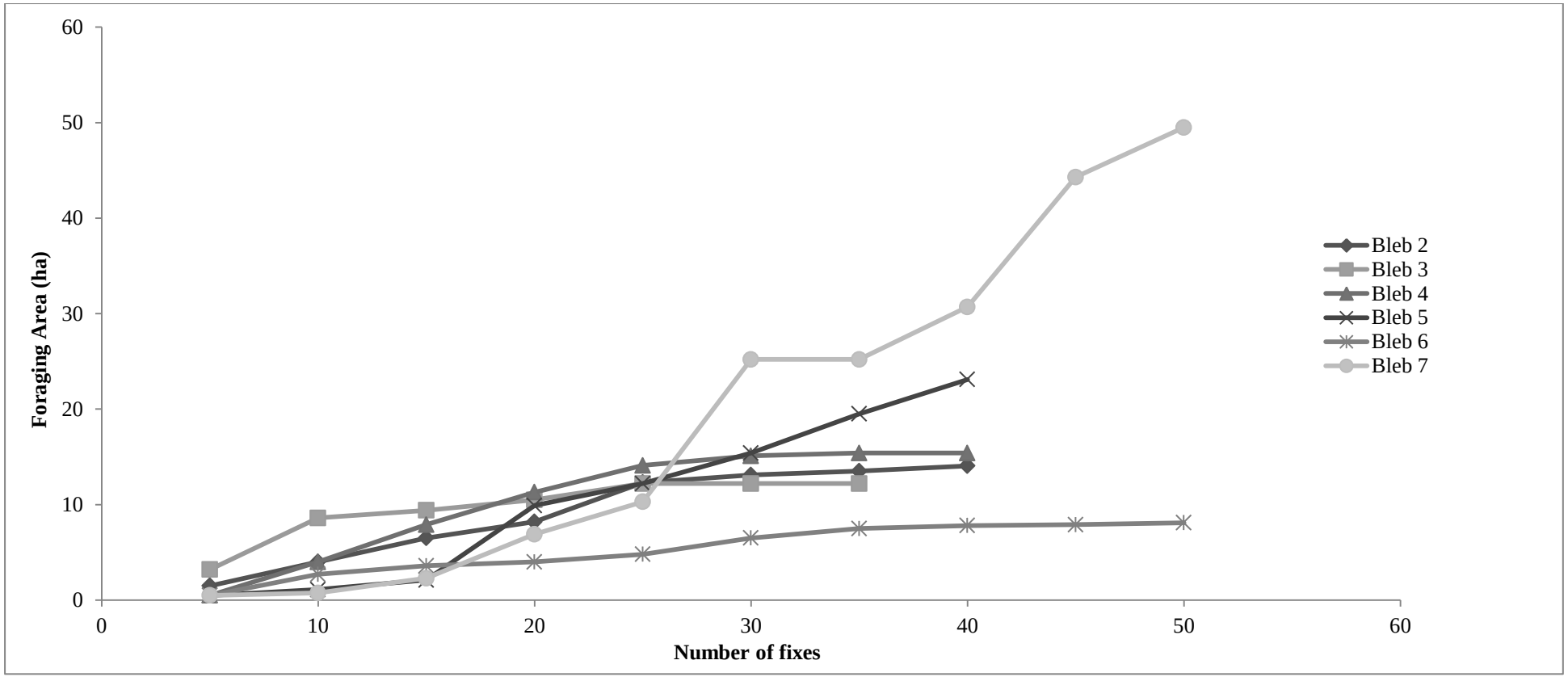


Figure 5.3: Cumulative home range size against number of successive locations for all *P.auritus* radio-tracked in July – Sept 2006. Estimates of range size are based on 100% minimum convex polygons.

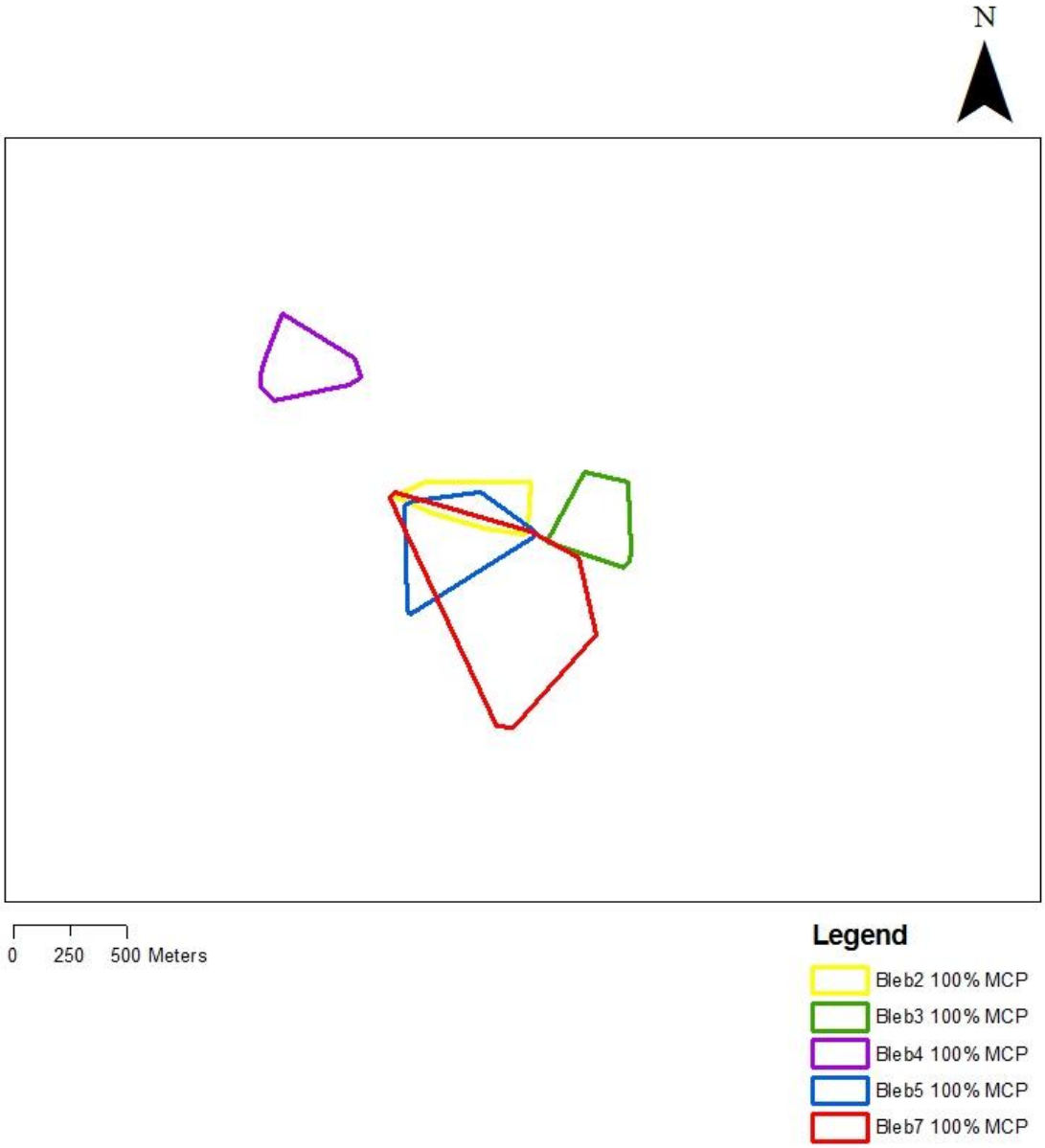


Figure 5.4: 100 % Minimum Convex Polygons home range representation of five *P. auritus* radio-tracked at Plashett Wood in 2006.

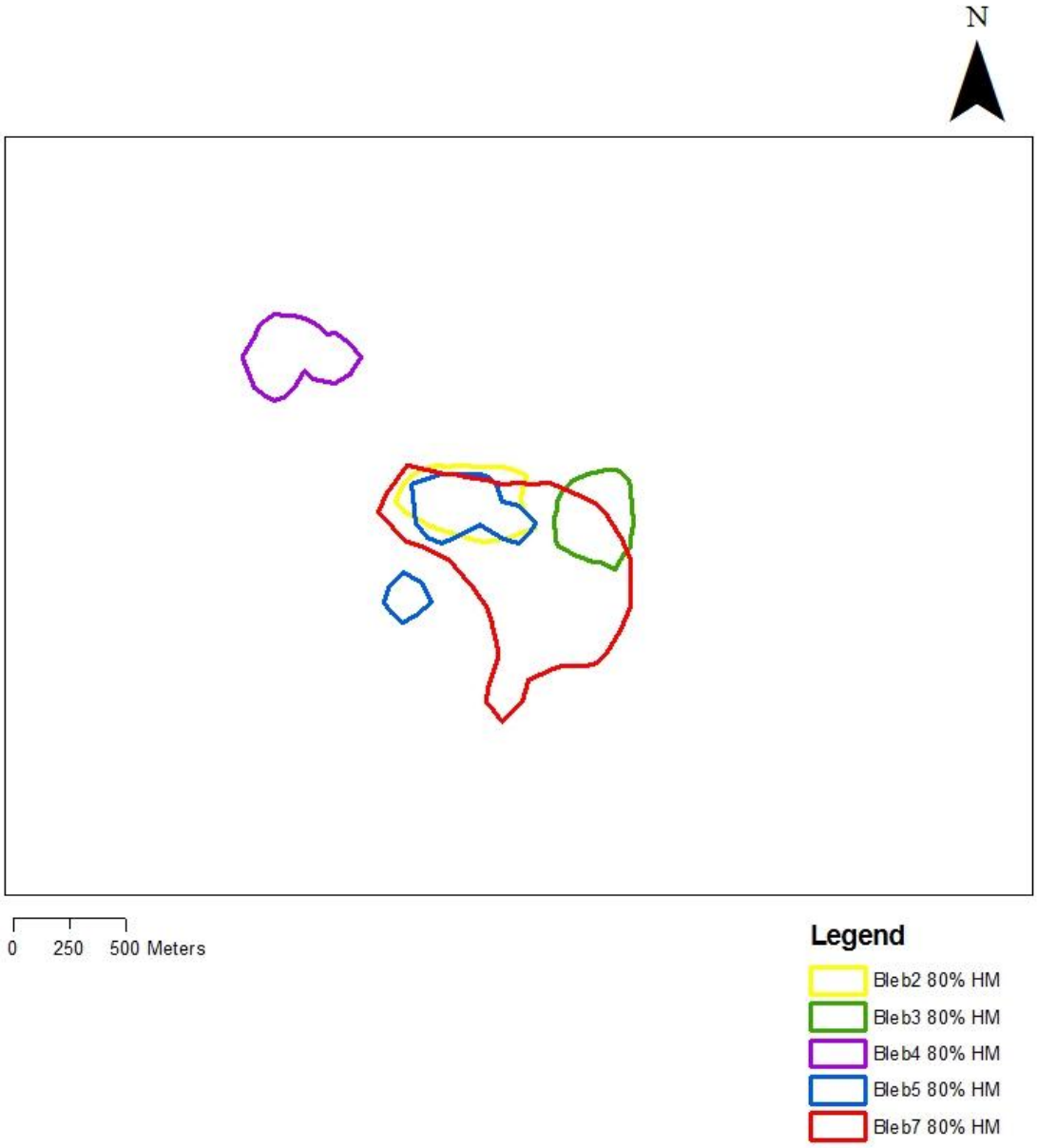


Figure 5.5: 80% Harmonic Mean home range representation of five *P. auritus* radio-tracked at Plashett Wood in 2006.

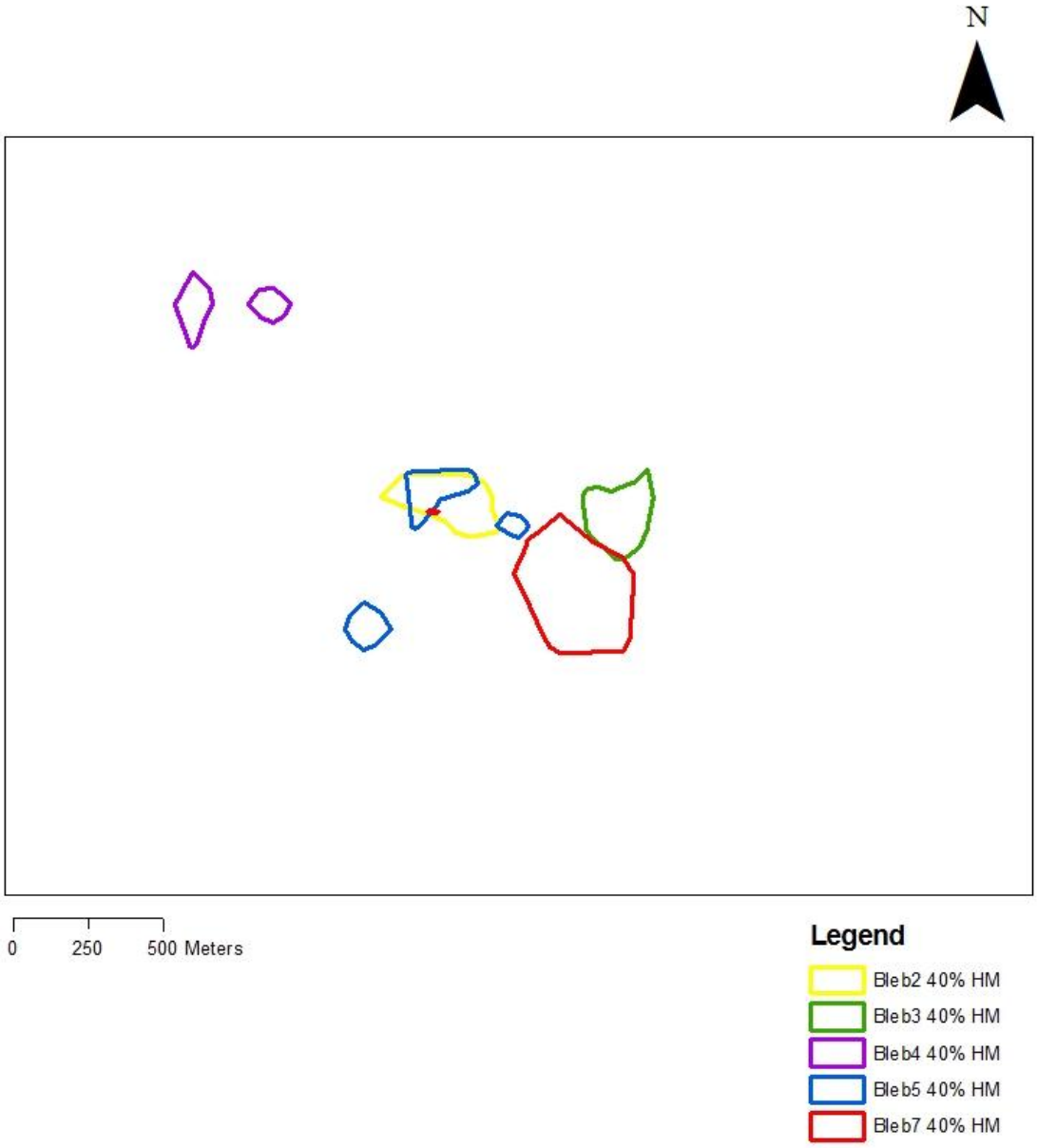


Figure 5.6: 40% Harmonic Mean home range representation of five *P. auritus* radio-tracked at Plashett Wood in 2006.

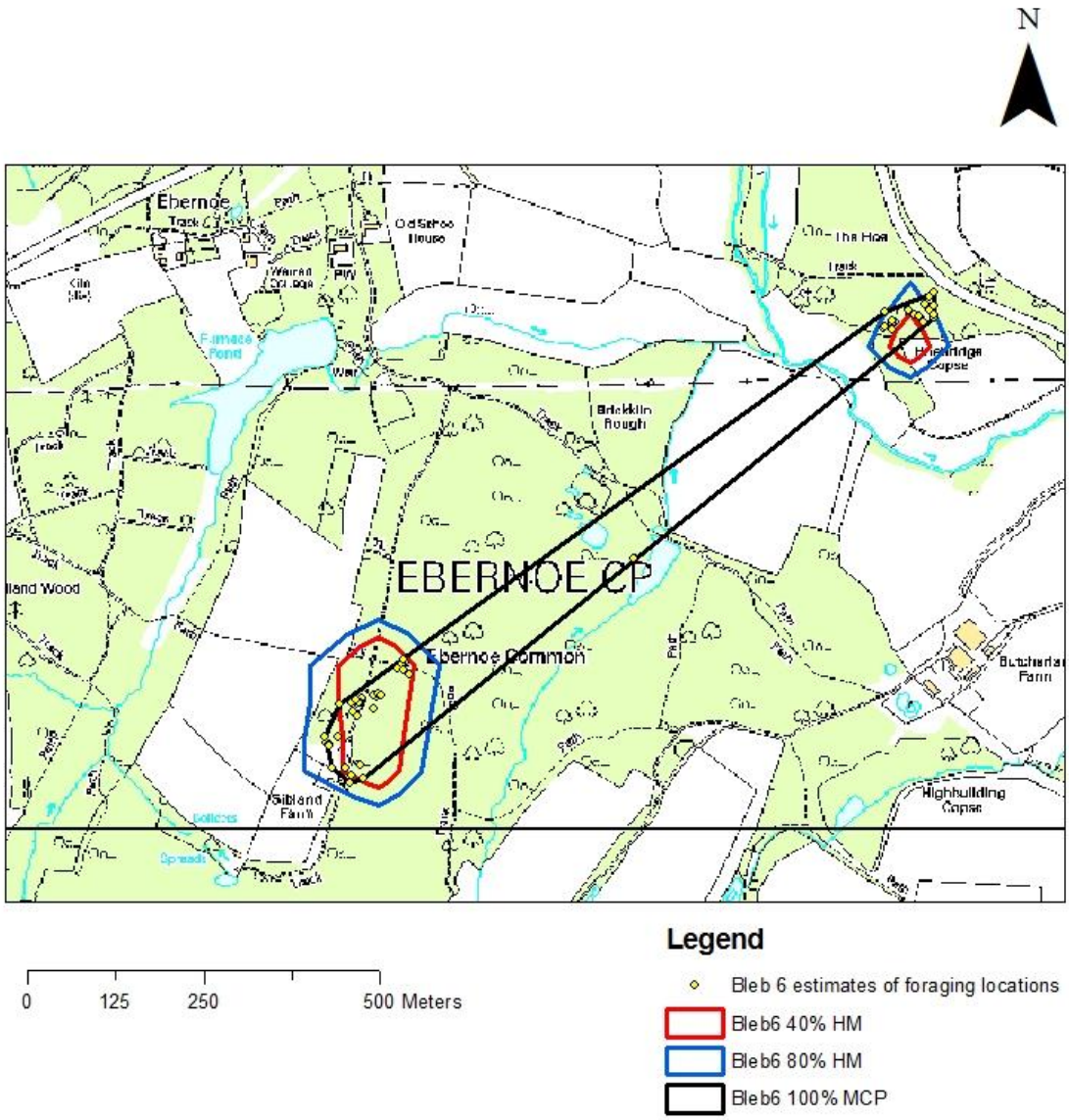


Figure 5.7: Bleb 6 radio-tracked at Ebernoe Common 2006. This female had two clear foraging locations, one in Ebernoe Common adjacent to her roost and a secondary foraging area in The Hoe approximately 1 km from her primary foraging area. The locations for many of her fixes were outside the 40%HM in her secondary foraging area, which illustrated that the home range analysis methodology may not be suitable.

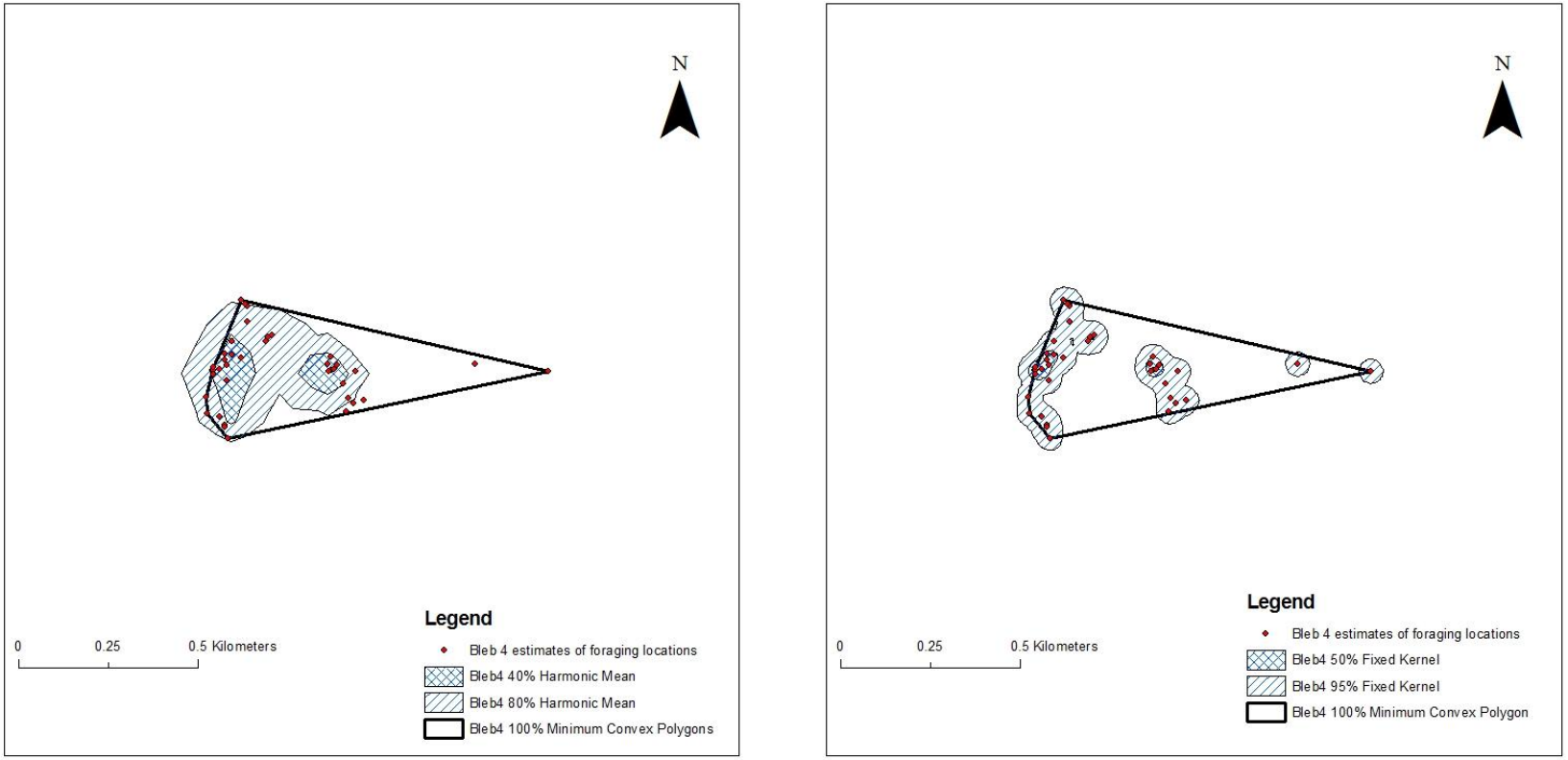


Figure 5.9: Comparative analysis of home range used for Bleb 4 using harmonic mean methodology (left) and fixed kernel estimated (right). The home range estimation of the 80% harmonic mean includes a major trunk road and an arable field situated between the two 40% harmonic mean core areas. These were habitats not frequented by bleb 4 and this is much more clearly represented by the fixed kernel analysis.

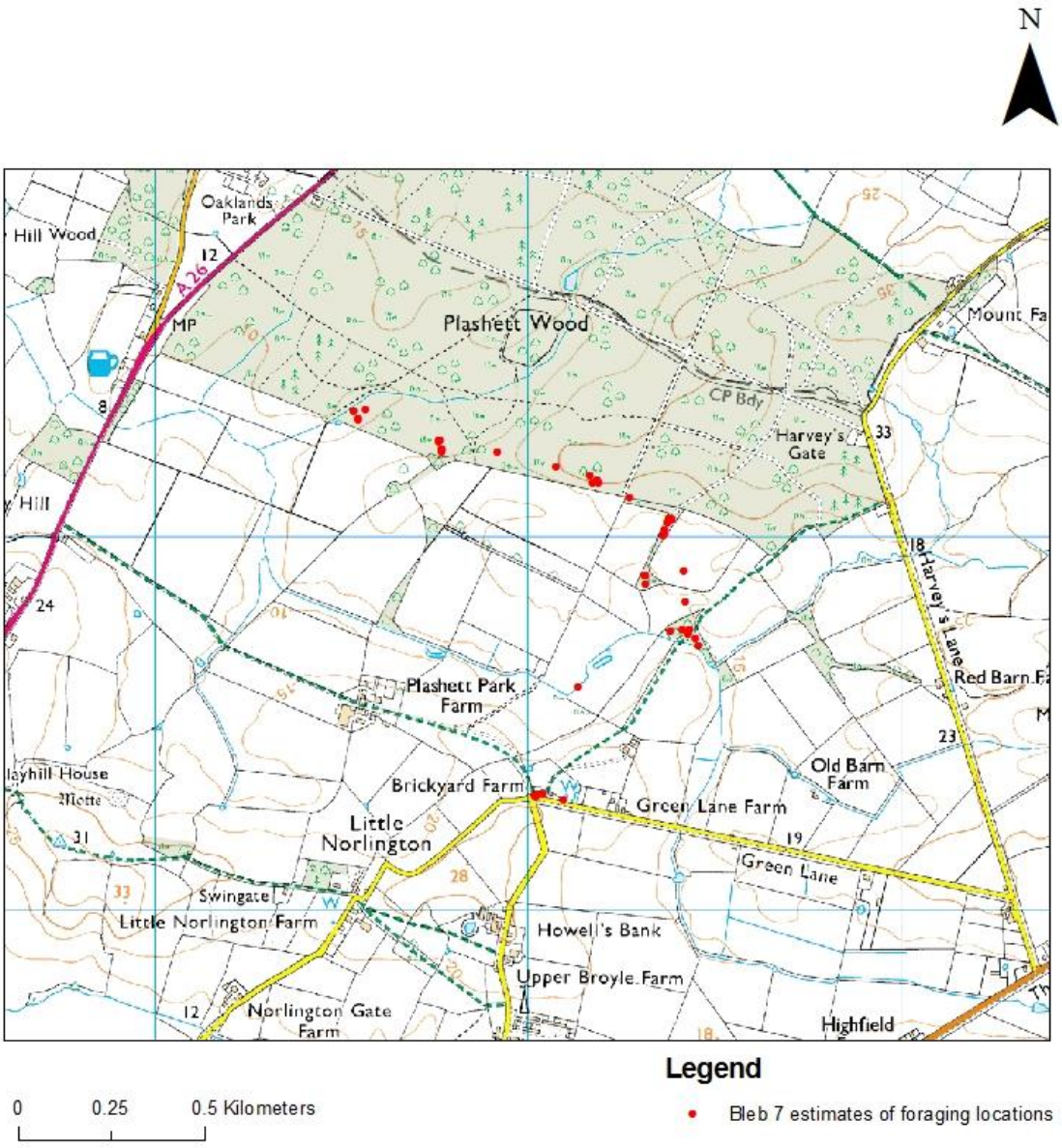


Figure 5.10: Estimates of foraging fixes for Bleb 7. The distribution of the fixes closely matches hedgerows, copses and woodland edge.

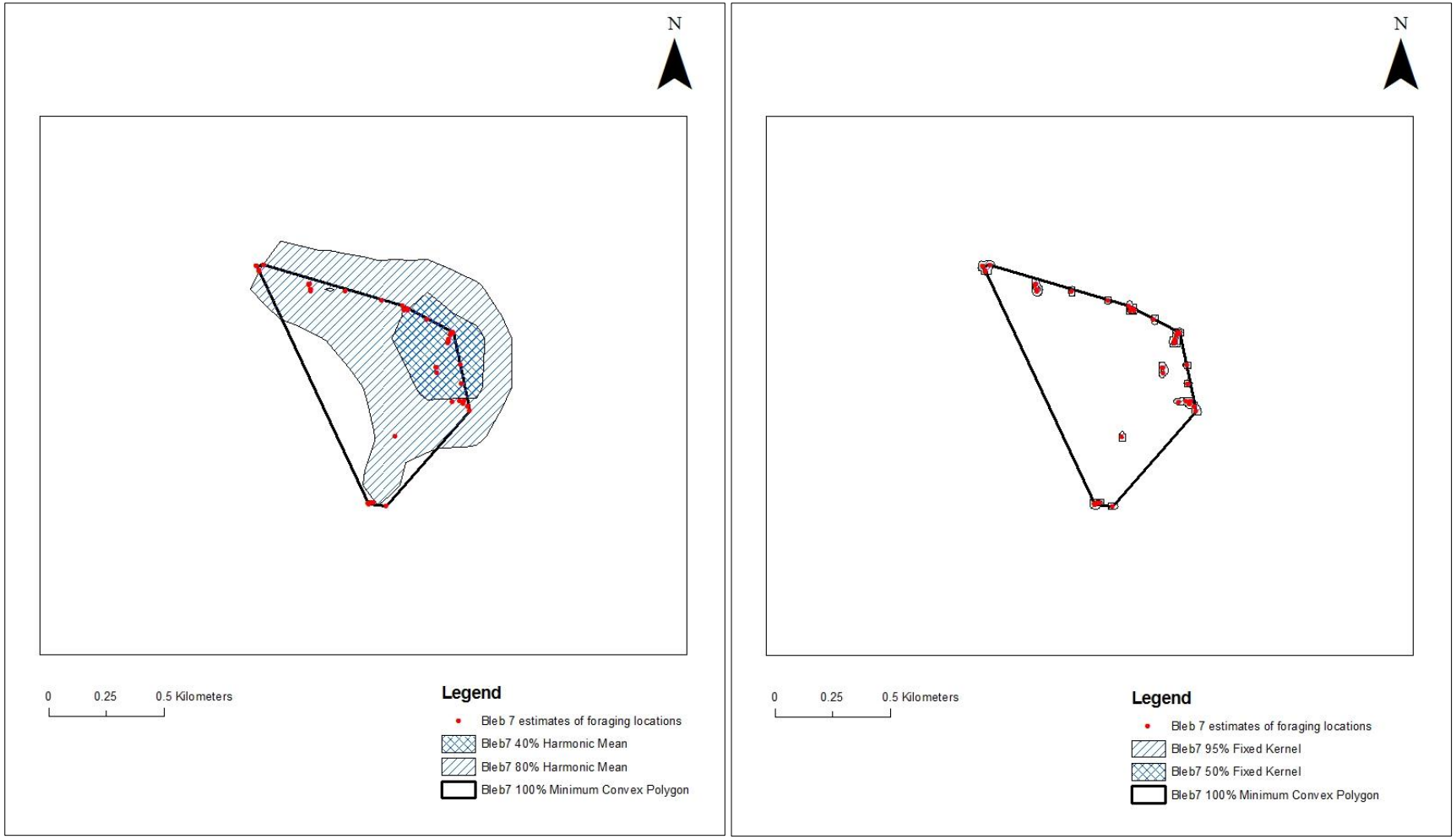


Figure 5.11: Comparative analysis of home range use for Bleb 7 using harmonic mean methodology (left) and fixed kernel estimates (right).

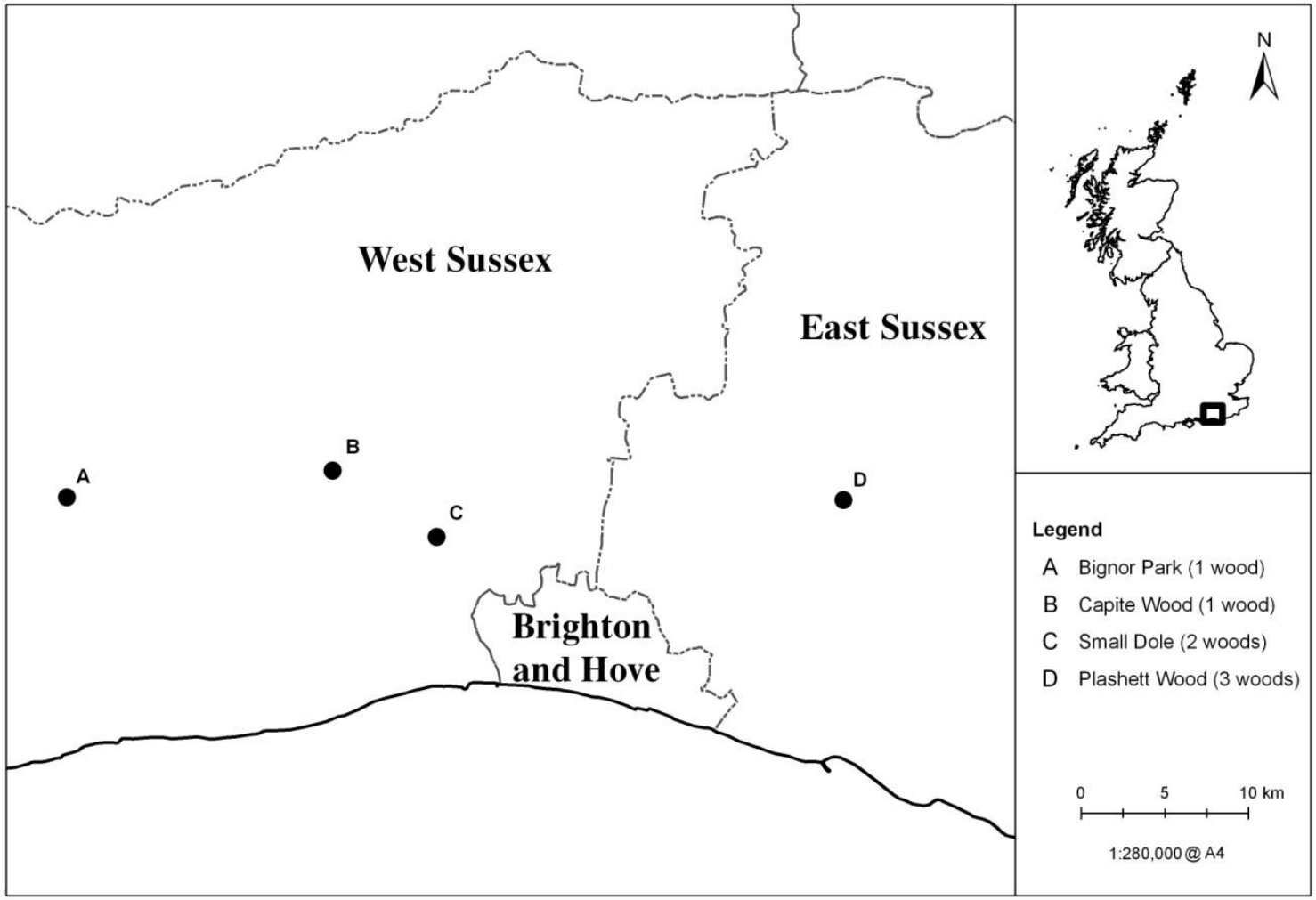
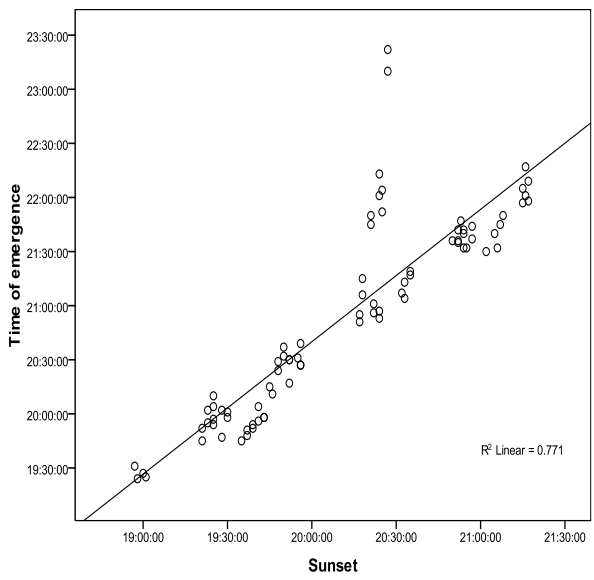


Figure 5.13: Locations of four sites (7 woodlands) where the radio-tracking study of *P. auritus* was carried out in 2007 and 2008.



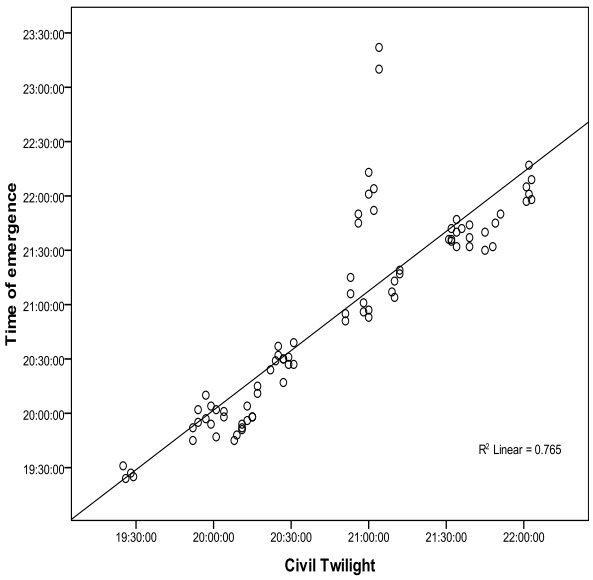
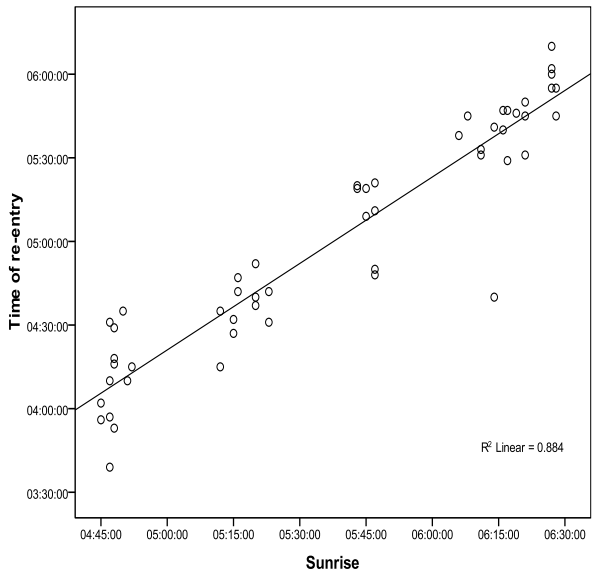


Figure 5.15a (left): Emergence time of radio-tagged female *P. auritus* was significantly correlated with the time of sunset.

Figure 5.15b (right): Emergence time of radio-tagged female *P. auritus* was also significantly correlated with the time of civil twilight.



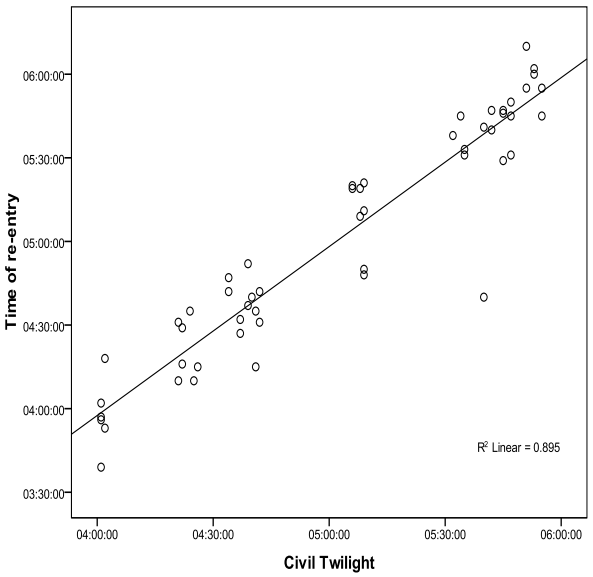


Figure 5.16a (left): Final roost re-entry time of radio-tagged female *P. auritus* was significantly correlated with the time of sunrise.

Figure 5.16b (right): Final roost re-entry time of radio-tagged female *P. auritus* was also significantly correlated with the time of civil twilight.

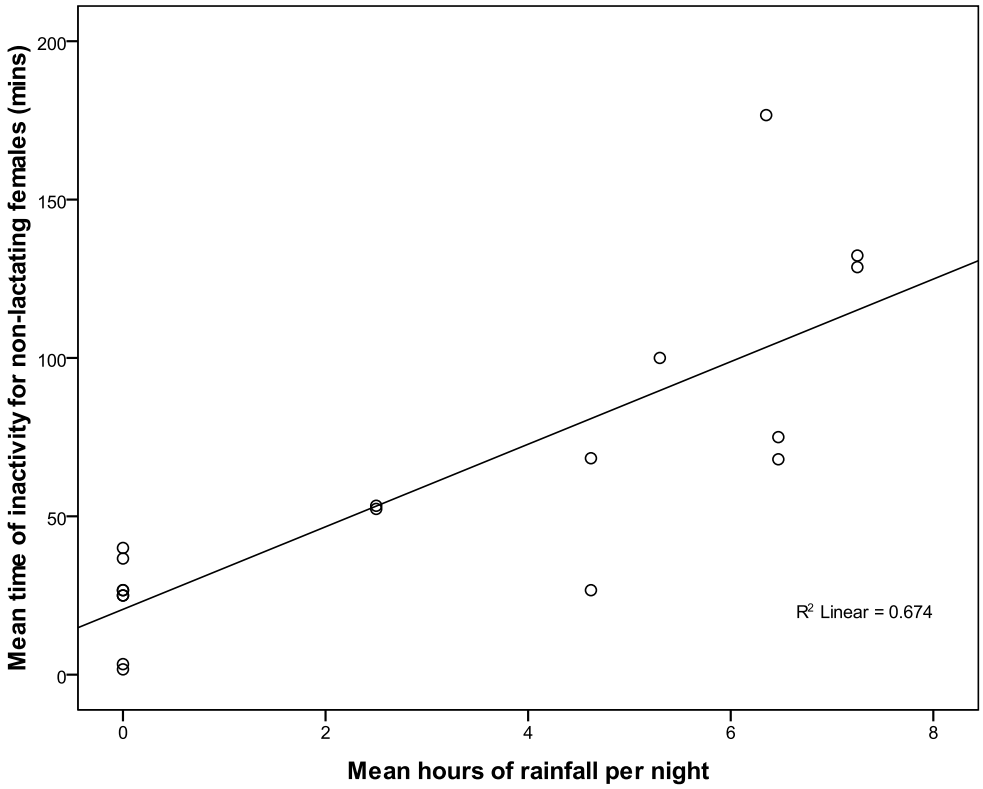


Figure 5.17a: Mean time spent inactive by radio-tagged non-lactating females was significantly correlated with the mean number of hours of rainfall per night.

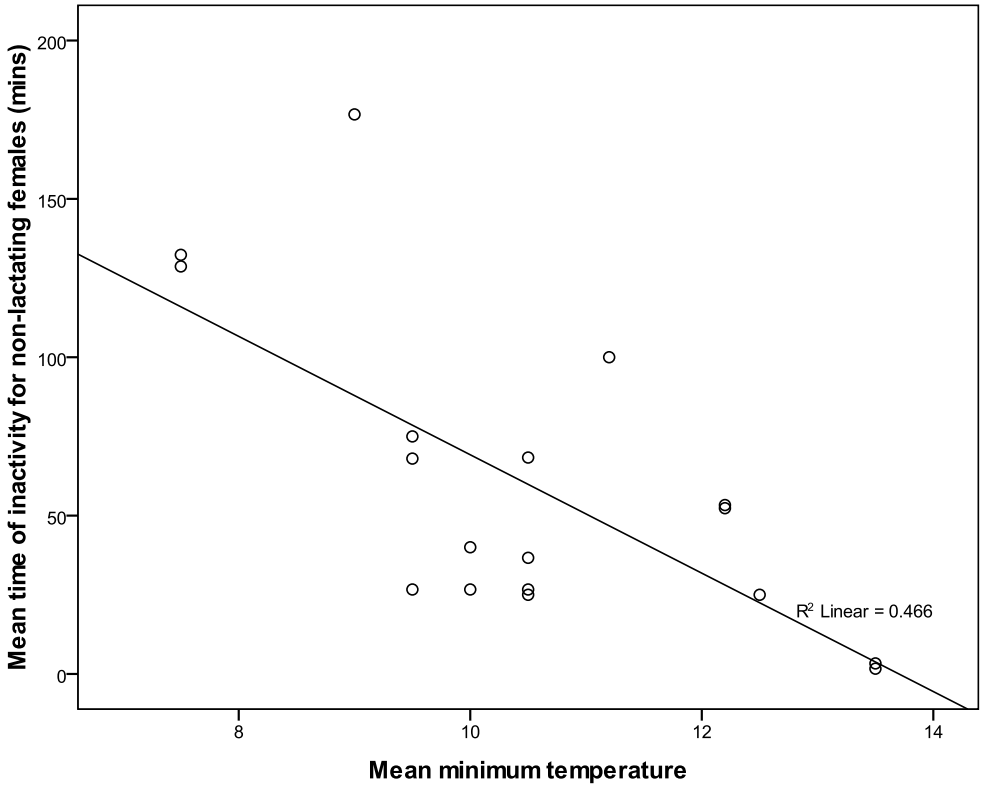


Figure 5.17b: Mean time spent inactive by radio-tagged non-lactating females was negatively correlated with the mean minimum temperature per night.

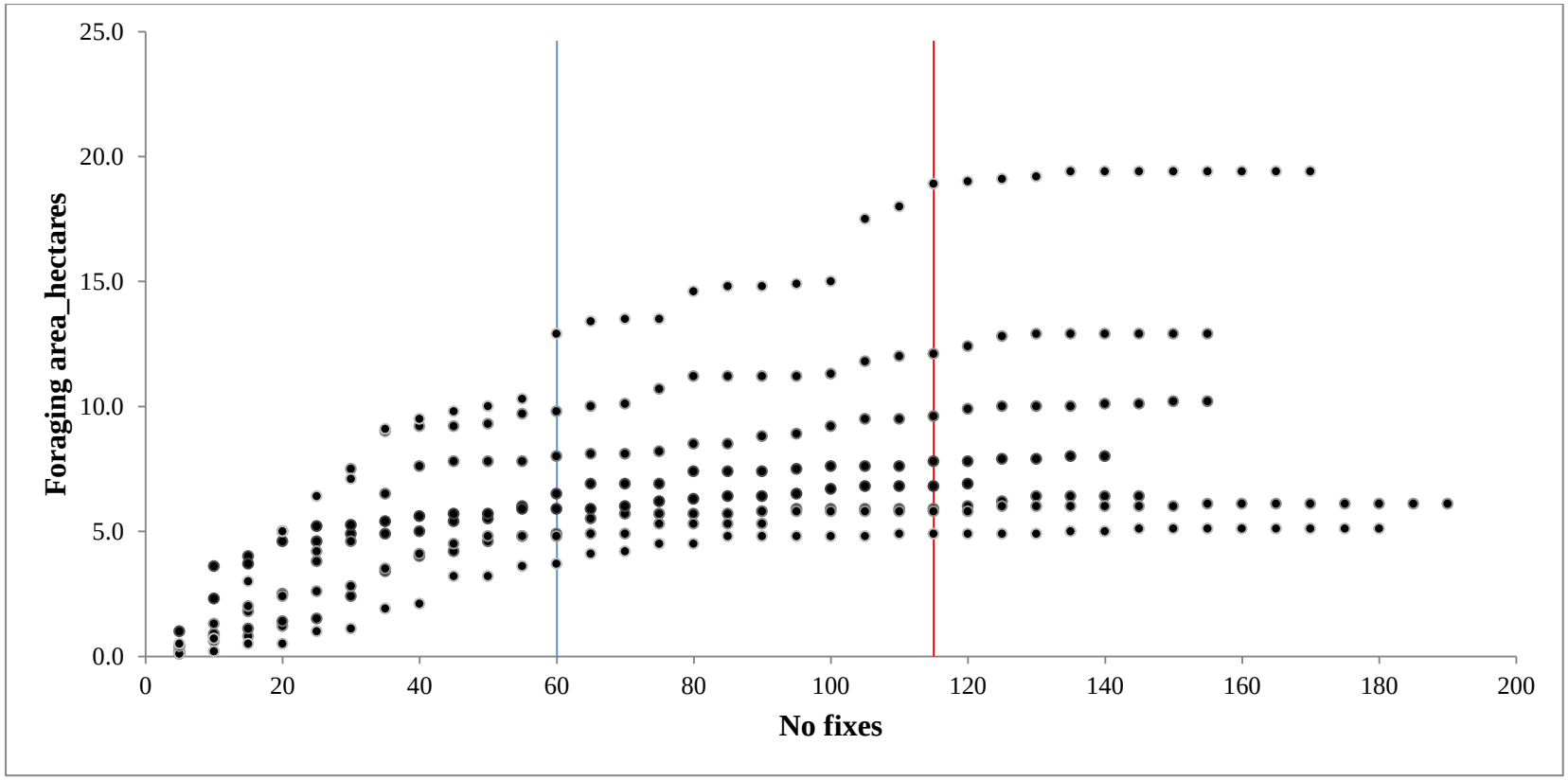


Figure 5.18: Cumulative home range size against number of successive locations of all female *P. auritus* radio-tracked in May – Sept 2007 (B8 – B15). The blue line shows the number of fixes whereby asymptote begins to start levelling off (ca. 60 fixes) and the red line indicates where asymptote begins to reach a stable estimate (ca. 115 fixes). Estimates of range size are based on 100% minimum convex polygons.

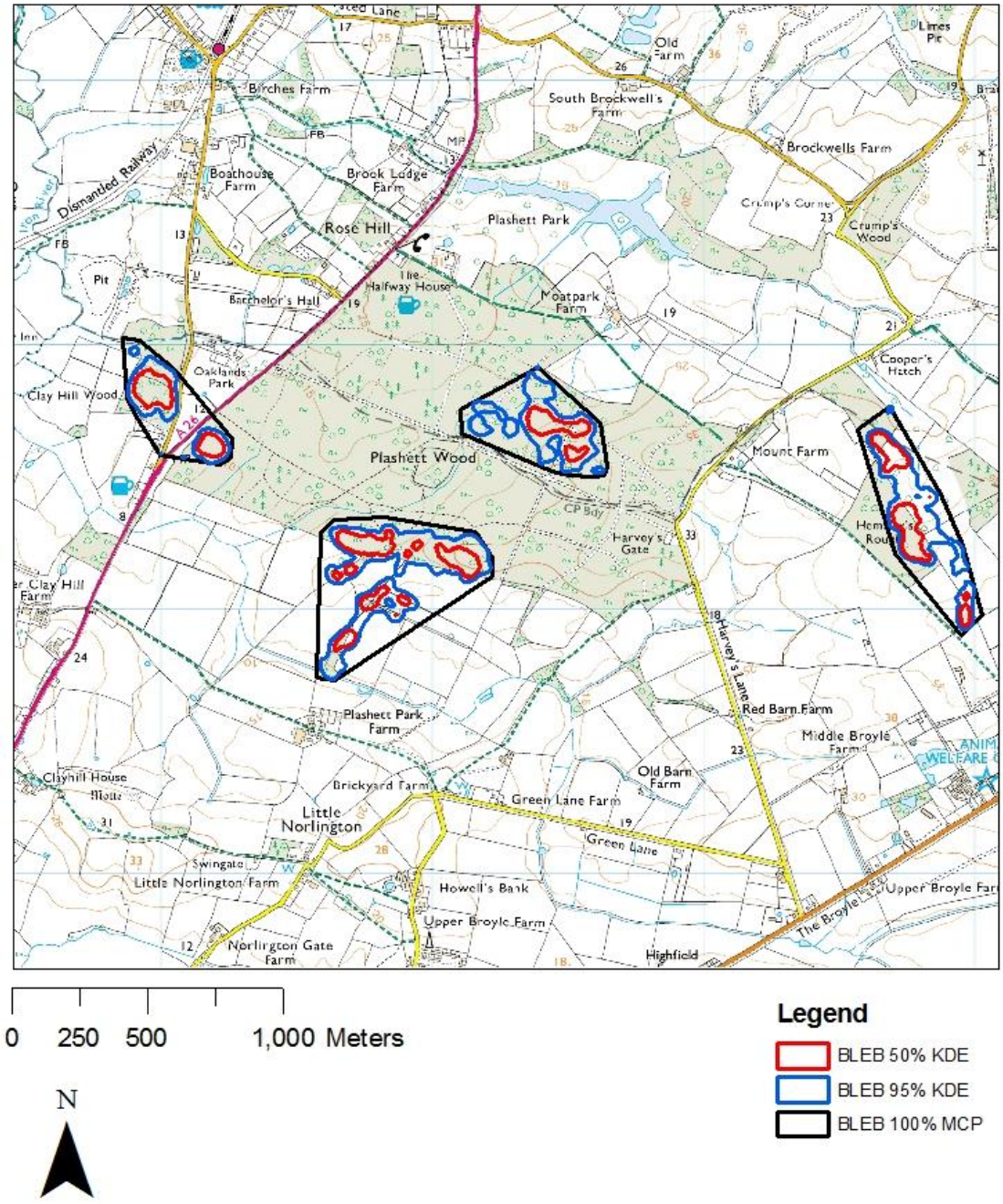


Figure 5.19: Spatial distribution of four individually radio-tracked bats in the Plashett Wood area in 2007. Individual home ranges are expressed as 100% MCPs enclosing all accurately recorded locations recorded. 95% KDE and 50% KDE reflect the distribution of the fixes, which are predominately associated with broadleaved woodland, copses and hedgerows.

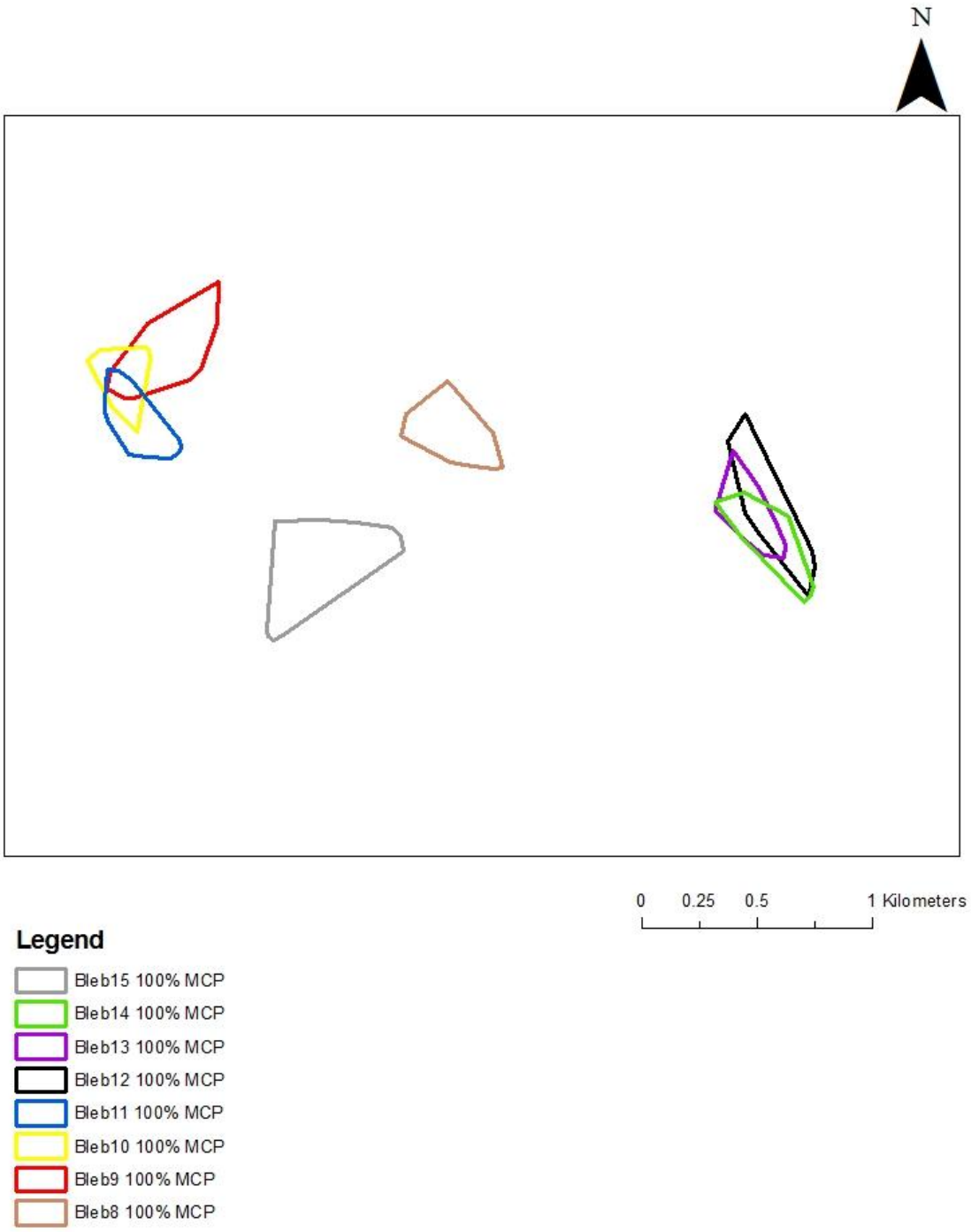


Figure 5.20: 100% MCP home range representation of eight females radio-tracked at Plashett Wood in 2007.

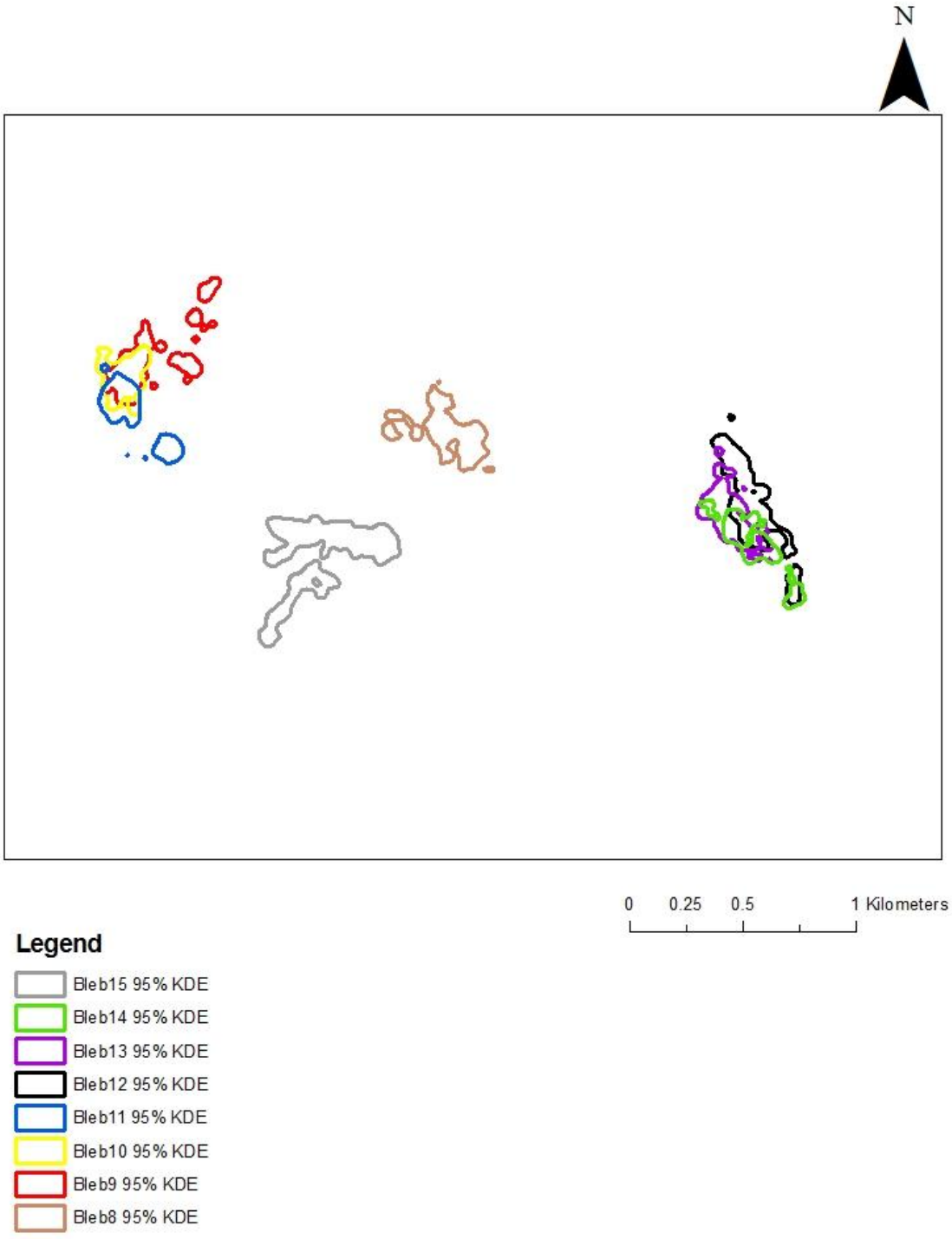


Figure 5.21: 95% KDE home range representation of eight females radio-tracked at Plashett Wood in 2007.

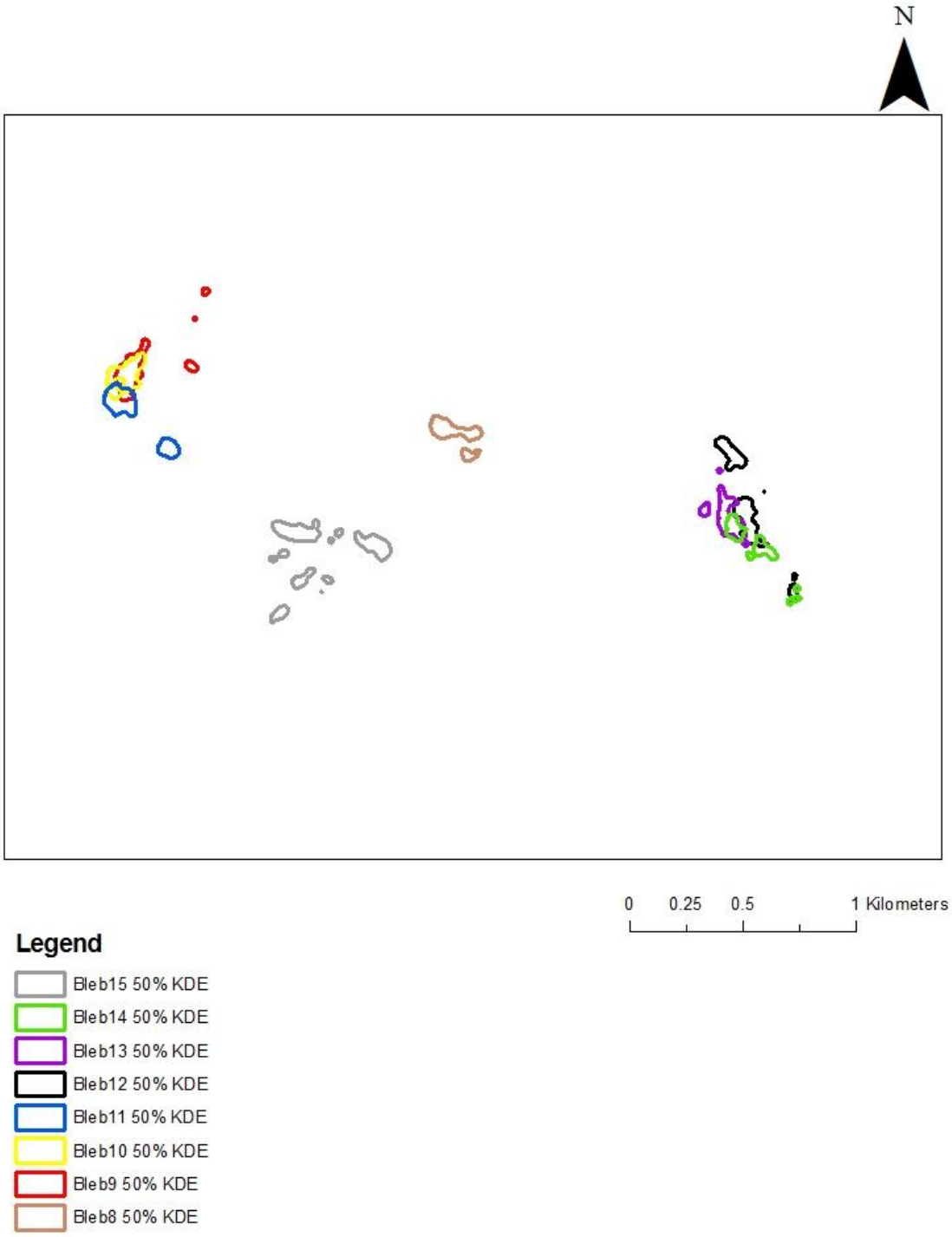


Figure 5.22: 50% KDE core foraging areas representation of eight females radio-tracked at Plashett Wood in 2007.

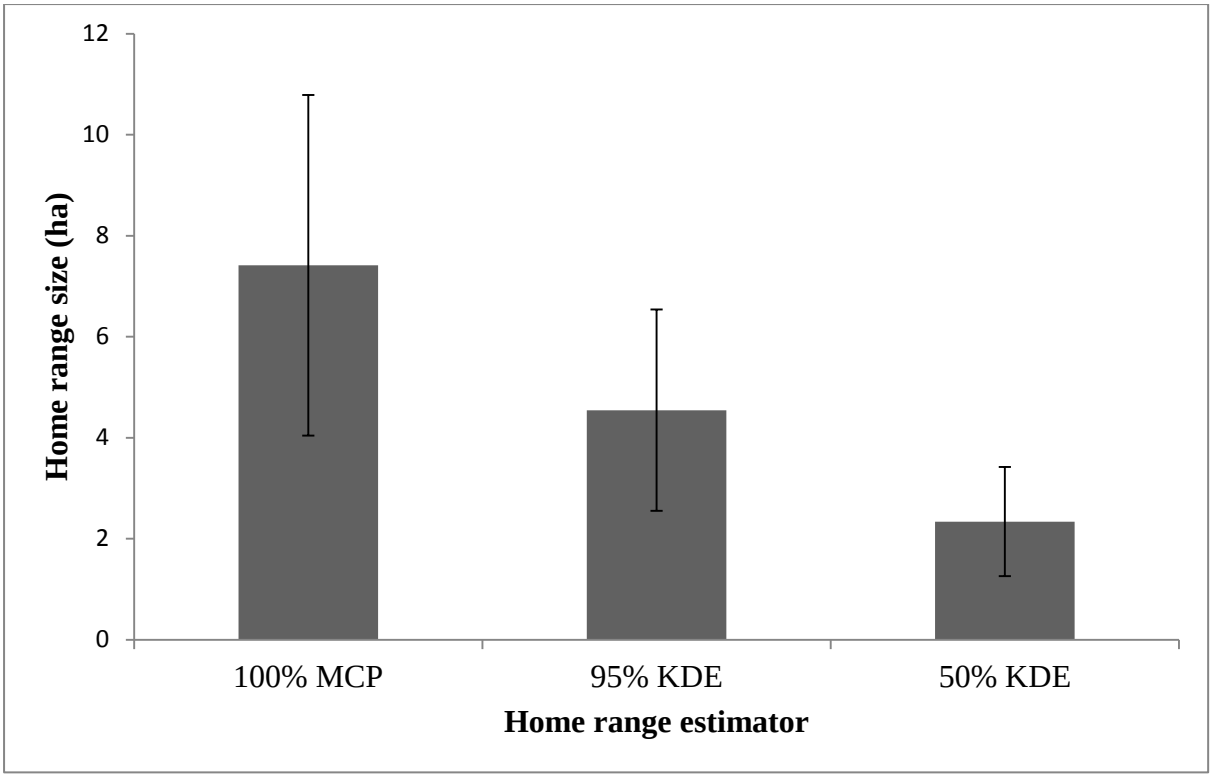


Figure 5.23: Mean ± standard deviation of home range size for 24 female *P.auritus*, radio-tracked from May – Sept 2007 and 2008. Estimates of range size are based on 100% minimum convex polygons, 95% & 50% kernel density estimators.

6.1 - Introduction

Territoriality as an attempt to maintain exclusive access to resources is a widespread phenomenon found in many animal species. In most species, however, actual fighting occurs far less frequently than one might expect from the occurrence of conflicts over territories and resources (Maynard-Smith and Harper, 2003). In mammalian behavioural ecology it is widely accepted that males compete for females (Dobson, 1982; Wolff, 1994) and females compete for resources such as food and shelter. Competition between female mammals for resources, other than mates, can have important reproductive consequences (Stockley and Bro-Jørgensen, 2010). The high energetic costs of gestation and lactation inherent in mammals (Loudon and Racey, 1987) means that the reproductive success of female mammals may be limited by access to resources such as food (Sterck et al., 1997; Stockley and Bro-Jørgensen, 2010) or nest sites (Hurst, 1987).

However, it has also been argued that female small mammals are territorial during the time of greatest food abundance and not during food limitation, which contradicts defence of resources hypotheses (Wolff, 1993; Wolff and Peterson, 1998). In which case alternative hypotheses, such as offspring defence, may better explain female territoriality in small mammals (Agrell et al., 1998). Not all female mammal species display territorial tendencies. Female territoriality is prominent among rodents, carnivores, insectivores, and some lagomorphs, but has been reported less frequently for bats, ungulates, marine mammals, marsupials, and most primates (Wolff and Peterson, 1998). It has been argued that in bats territoriality is extremely unlikely to occur in species that rely on a patchy sources of food that undergo large seasonal or unpredictable changes (Bradbury, 1977) and, in such

circumstances, group foraging may be a more optimal strategy. Nonetheless, it has been found that for other species of bats there is a tendency for females to use small individual foraging areas in pregnancy and lactation, for example in species such as Daubenton's bat *Myotis daubentonii* (Dietz and Kalko, 2007) and Bechstein's bat *Myotis bechsteinii* (Kerth et al., 2001; Dietz and Pir, 2009), and it is entirely plausible to suggest that some mechanism must exist in order for females to maintain their access to such a crucial resource, particularly when energy demands are at their greatest.

One mechanism for repelling intruders from a feeding territory is the advertisement of ownership by means of vocal communication (McComb and Reby, 2009). However, unlike species that emit social calls regularly at foraging sites, such as pipistrelle bats (Barlow and Jones, 1997; Russo and Jones, 1999), the emission of social calls away from the roost is a sporadic and infrequent event for *P. auritus*. The actually frequency is unknown, but it seems likely from continuous recording that bats typically call only a few times per night. Given this low frequency it would be extremely difficult to study the function of social calls by field observation and recording alone. Therefore, as an alternative approach, it was decided to investigate aspects of the function of social calls at foraging sites by examining how bats respond to playback rather than trying to investigate the conditions under which they are emitted).

There have been a number of functions proposed for the social calls of bats, given in differing contexts away from the roost, including: (i) defence of resources in pipistrelle bats (Racey and Swift, 1985; Barlow and Jones, 1997); (ii) attraction of mates in little brown bats *Myotis lucifugus* (Barclay and Thomas, 1979) and greater sac-winged bats, *Saccopteryx bilineata*

(Behr and von Helversen, 2004); (iii) group cohesion in spear-nosed bats *Phyllostomus hastatus* (Wilkinson and Boughman, 1998).

Chapter 4 detailed how *P. auritus* bats respond to synthesised social calls by rapidly approaching the source. Male and female *P. auritus* were caught using simulated social calls throughout the year, not only in the mating period, so the mate attraction hypothesis seems not to account for why *P. auritus* responded to the simulated social calls. The group cohesion hypothesis also seems unlikely to explain why individuals responded, as the response was often rapid and would sometimes appear aggressive (see Chapter 4). Therefore, defence of resources would appear to be a more plausible alternative explanation and, if true, it would be expected that there would be a differential response in relation to where the stimulus is located within a bat's foraging range. If a female *P. auritus* response to the stimulus represents defence of resources within their foraging territory, it would be expected that a female would be more responsive to the stimulus within her foraging range than outside of it.

Chapter 5 detailed how the home range for individual females was defined. Radio-telemetry provides a technique of tracking known individuals and providing estimates of location. However, ascertaining whether the focal radio-tagged bat has or has not responded to the stimulus is more problematic. This is because the transmitters are not always visible on infra-red cameras due to their small size and the fast fluttering flight of bats. Using telemetry to estimate the bat's position in relation to the stimulus would not provide enough rapid or precise data, especially considering the way in which *P. auritus* respond to the stimulus as

detailed in Chapter 4 (often a quick short responses). Therefore, in order to accurately identify the focal individual responding to the stimulus, it was essential to develop and test novel research equipment. An estimate of the distance from which *P. auritus* reliably responded to the stimulus was also required.

Section 6.2 details preparatory work in which three studies were conducted in order to develop and test novel research equipment. The first study describes the development of a system to register a close response of a radio-tagged individual, termed the 'LocateABat' system. The second study tests whether radio-tagged bats responded to the stimulus and, if so, whether the 'LocateABat' system registered an appropriate response. The third study describes a short experiment with captive bats to test their ability to hear the stimulus at differing distances under field conditions. Section 6.3 describes an experiment to test the hypothesis that a female's response to the stimulus represents defence of resources in relation to range use.

6.2: Preparatory Work & Pilot Studies

6.2.1: 'LocateABat' System

6.2.1.1 - Introduction

A system to detect when a radio-tagged bat approached the stimulus was designed and custom-built by Peter Reed of the University of Sussex. This detection system, termed 'LocateABat', was designed to sense when the signal of the radio-tag attached to the focal bat was within a given range. If the focal bat responded to the stimulus produced by the Autobat by approaching, then the 'LocateABat' system should also register a near response.

6.2.1.2 - Methods

6.2.1.2.1 - The LocateABat system design

An omni-directional antenna was attached to a Sika receiver that was set to the frequency of the radio-transmitter. The audio output of the Sika receiver was fed to a microprocessor-based circuit that measured the signal strength and converted this to a numerical value. This was output from the circuit via an RS232 serial data link to a laptop running a Visual Basic program that then displayed this value as a number on the screen. The value was also written to a file on the laptop along with the current real time. These values increased as the radio-transmitter approached the omni-directional aerial.

6.2.1.2.2 - System calibration

The output of the receiver was calibrated to produce five volts from the interface box when the transmitter was two metres away. The software on the laptop measured this voltage and displayed it as a reading (where 5V equated to a reading of 1000). As the radio-transmitter moved away from the antenna, the signal level dropped, the five volts dropped, and the value of 1000 dropped, proportionally. A field test was conducted to determine whether the 5V value of the radio-transmitter (which was set at two metres from the omni–directional aerial) dropped proportionately with increasing distance. The accuracy of the readings was dependent upon the proximity of the receiver to the transmitter, and this affected the accuracy of the estimates. Furthermore, topographical features or buildings commonly deflected signals or caused other interference (Priede, 1992; Parker et al., 1996) which would also influence the accuracy of the estimates.

Therefore, two experimenters (Stephanie Murphy and Peter Reed) tested the accuracy of the system in an open field (where topographical features or buildings were unlikely to interfere with the accuracy). A radio-transmitter was clipped to a piece of rubber tube and the tube was held by the first experimenter (to avoid the transmitter signal being affected by human contact). The first experimenter stood at a distance of two metres, at 180^{o}, from the omni directional aerial, and the volt meter (Tenma model 72-6260 Digital multi-meter) was set at 5 volts DC by the second experimenter. The first experimenter successively moved to a distance of 5, 10, 15, 20 and 25 metres away at 180^{o}, and the voltage registered on the volt meter was recorded by the second experimenter at each interval. This process was repeated a further twenty times in the opposite direction to ensure that the measures that were taken were comparative in different directions. The entire process was repeated in broadleaved deciduous woodland, the habitat type most frequently associated with *P. auritus* foraging (Swift and Racey, 1983; Entwistle et al., 1996); Murphy *et al* in prep.), in order to ascertain whether the system could produce reliable estimates of distance in the common foraging habitat of *P. auritus*. The consistency of the output was analysed using test/retest reliability (which calculates the variability of the resulting outputs) to compare the results of each test. The correlation coefficient between sets of results was used as a quantitative measure of the test-retest reliability using SPSS 17.0 (©SPSS Inc).

6.2.1.3 - Results

The results of the field trial studies revealed that the LocateABat system detects with 95% confidence when the radio-tag is within 10 metres of the omni directional aerial within broadleaved woodland (i.e. cluttered habitats as detailed in Table 6.1). Distances in excess of

10m were unlikely to be reliable, however, as there was too much variation from the readings registered, as indicated by the weak correlation between the test and the re-test. The accuracy of the detection rate improves in open areas, as detailed in Table 6.2, but as the experiments for testing *P. auritus* responses to the Autobat were to be carried out in broadleaved woodland, a conservative estimate of 10 metres was applied.

6.2.1.4 - Conclusions

The results of the pilot study, testing the accuracy of the LocateABat system, indicated that the methodology would be functional in detecting a radio-transmitter on a radio-tagged bat at a short range distance of up to 10 metres from the receiver.

6.2.2 – Trialling the LocateABat system

6.2.2.1 – Introduction

Once the LocateABat system had been developed and field tested using radio-transmitters, it was vital to ascertain whether the system would accurately register the radio-transmitters once attached to a bat. Moreover, it was essential to determine whether *P. auritus* would continue to respond to the stimulus once they had been radio-tagged (as the previous encounter with the stimulus had resulted in the individual being trapped, handled and a radio-transmitter attached, a negative experience that may induce 'response shyness'). Studies on vertebrates, such as the snapper fish, *Pagrus auratus*, found evidence that tagging induced trap shyness and tagged fish had a reduced probability of recapture by the method by which

they had originally been caught (Gilbert et al., 2001). Similarly, camera trapping studies on tigers, *Panthera tigris*, in India found that some individuals became trap shy (probably as a result of being scared by the flash) and capture rates decreased by more than 50% after the first five days of trapping. Studies on avian biology have also found that mist nets can be particularly inefficient in recapturing previously captured birds (Macarthu.Rh and Macarthu.At, 1974; Macarthur and Macarthur, 1974). However, not all vertebrates actively avoid traps as studies on badgers, *Meles meles*, have shown that some individuals are positively oriented toward being trapped (Tuyttens et al., 1999). The trappability of North American red squirrels *Tamiasciurus hudsonicus* was context dependent and positively correlated with their activity levels. A propensity for the squirrels to enter traps was associated with increased activity, intrusion on the territories of others, and moving farther from their own territory (Boon et al., 2008).

Therefore, the objectives of this study were to: (i) assess the practicality of the Locatabat system when used with transmitters on free-flying bats; (ii) ascertain whether radio-tagged bats, previously captured and tagged using the Autobat acoustic lure, would respond again; and, also (iii) test the feasibility of altering the stimulus location in relation to home range use.

6.2.2.2 - Methods

Six bats (five female and one male) were radio-tracked and their subsequent estimates of location analysed using the harmonic mean (HM) methodology (see Chapter 5 Section 5.2 for detailed radio-tracking study). For each bat, once sufficient data had been gathered to determine its range use over three successive nights, the experiment was set up in the 40% HM core foraging range. Data for two bats (one male, bleb 5, and one female, bleb 7) did not reach asymptote, indicating sufficient data had not been collected. However, it was decided to terminate radio-tracking to enable the trial experiment to commence (this being the primary focus of the pilot experiment). Poor weather conditions meant that extended periods of inactivity for these individuals occurred and there was the potential for the radio-transmitter to fail prior to the trial experiment being completed.

6.2.2.2.1 - Location of stimulus

The location chosen for the stimulus to be situated in the core area was within the area visited most frequently during the radio-tracking period, in the 40% HM. The location chosen for the stimulus to be situated within the peripheral foraging area (but excluding the 40% HM) was based upon locations visited less frequently (but nonetheless frequented) during the tracking period. In order to test whether the bats would respond to the stimulus outside of the previously determined home range, a location was chosen for the stimulus, in suitable *P. auritus* habitat, adjacent to the each focal bat's foraging range, but in a location at which they had not been previously recorded.

6.2.2.2.2 - Trial experiment

The Autobat speakers were suspended from a horizontal tree branch, using hooks, at a height of approximately three metres. Two Sony Handycam (Model DCR – DVD91E) digital video camera recorders were set up to film the bats responses to the Autobat. One camera was placed directly underneath the speakers filming vertically and the second camera was position approximately three metres away from the speakers filming horizontally. The camera filming the horizontal view filmed the focal bats behaviour as it approached the stimulus, but was not a reliable estimate of how close the bat approached. For this, a combination of horizontal and vertical cameras was necessary to film the bat from two different angles. Two infra red lights (Model Envin micro 75L) were used to illuminate the area around the speakers. The omni-directional aerial of the LocateABat system was placed near to the speaker to detect the approaching radio-tagged bat. A synthesised social call, call A (see chapter 4), was then produced by the Autobat and the bats that responded to the call were captured on film. The radio-tagged bats that responded also registered on the LocateABat system. Their vocal responses were recorded using a Petterson D240x bat detector and a Sony portable MD recorder (Model MZ-RH710). The Autobat broadcasted call A for a period of five minutes with an interval of five minutes of silence. After a period of one hour the process was terminated and repeated at the periphery and the exterior of each bat's foraging range. The position of the bat within its range was estimated at least every five minutes during the experiment (where possible) by a surveyor taking estimates of locations by radio-tracking.

6.2.2.3 – Results

6.2.2.3.1 - Responses of radio-tagged P. auritus *to synthesised social calls*

All five female bats responded to the Autobat when played in their core foraging range (40%HM). The mean number of responses for each female was 2.2 per hour. Two female bats responded when the stimulus was played in the peripheral range, and one female bat responded when the stimulus was played outside of her range. All confirmed responses of radio-tagged bats were captured on film and the output of the LocateABat system registered a peak in voltage concurring with the time of the filmed response (meaning we could be 95% confident that it was the focal bat responding within the five metre range). The radio-tracked male bat did not respond to the Autobat in either his core or peripheral range nor outside of his foraging range.

6.2.2.3.2 - Estimates of the distances radio-tagged bats responded

The estimates of locations, obtained from the radio-tracking data during the experimental period, were calculated and the distance (to the nearest metre) from the stimulus location to each estimated bat location was calculated. Each of the five female bats that responded to the stimulus in their core foraging range were located a mean distance of 65 metres (minimum 30; maximum 115) from the stimulus when the experimental began. The mean estimated distance of the five females from the stimulus in the peripheral foraging range was 175 metres (minimum 65; maximum 280) and, at the exterior of the foraging range, 300 metres (minimum 85; maximum 450). Table 6.3 details the number of times each radio-tagged bat responded to the Autobat, how close each bat approached to the stimulus and, also, the mean estimated distance that each bat was located from the stimulus during the pilot study.

6.2.2.4 – Discussion

The individuals that responded were recorded on infra-red video and a simultaneous response registered on the LocateABat system, which demonstrated that the LocateABat system was satisfactory for registering close responses of free-flying bats to the stimulus. All five radio-tagged female bats responded to the stimulus when it was played in their core foraging range (40%HM), with a mean of 2.2 responses per hour. Two female bats responded when the stimulus was played in the peripheral range, and one female bat responded when the stimulus was played outside of her range. The number of individuals responding, and the fact that some individuals responded on more than one occasion, indicates that the previous experience of being captured and radio-tagged was unlikely to deter motivation to respond to the stimulus.

It was considered that the differences in the response of *P. auritus* to synthesised social calls may have been influenced by the position within the bat's foraging area (i.e. core or peripheral) that the stimulus was located. However, the experiment needed to be carried out in the core, peripheral and exterior foraging range of each bat at a distance where it was known that the focal bat could hear the stimulus. Only one of the six radio-tagged bats responded to calls from the exterior of their range. However, based on radio-tracking data, the mean distance of the pilot study bats from the stimulus in the exterior location during the experimental period, was approximately 300 metres (SD = 127, n=5). By comparison, the mean distance of the pilot study bats from the stimulus in the core location during the experimental period, was 67 metres (SD = 34, n = 5). In order to test whether an individual's response was influenced by where in their range the stimulus was broadcast, a comparable

distance from the focal bat to the stimulus was required for each range location. This was to ensure that a result of 'no response to the stimulus' would mean that the bat heard and ignored the stimulus, as opposed to the focal individual not hearing the stimulus and, as a consequence, not being motivated to respond. Further analytical work on the distances *P. auritus* could hear the stimulus from was required in order to construct a robust experimental system to test whether responses to synthesised social calls are influenced by where the stimulus is located within their home range.

6.2.3 – Determining the most reliable distance from which *P. auritus* are likely to respond to the stimulus.

6.2.3.1 – Introduction

Social systems largely depend on communication among group members and the receivers' ability to assess the position and identity of the signallers (Bradbury and Vehrencamp, 1998). For effective acoustic communication to occur, an emitted signal must reach a receiver with enough clarity to allow an appropriate behavioural decision to be made (Arch and Narins, 2008). High frequency sounds attenuate more rapidly with distance (Morton, 1975; Lawrence and Simmons, 1982; Romer and Lewald, 1992; Morton, 1998) and, therefore, it would be expected that animals engaging in long-distance communication, or communicating in cluttered environments, would concentrate their vocal efforts by using low frequency sounds to ensure that their calls have a higher probability of reaching their intended target.

Although studies have demonstrated supporting evidence for the importance of nocturnal interaction among potential mates or group members (Barclay, 1982; Boughman and Wilkinson, 1998; Wilkinson and Boughman, 1998; Rossiter et al., 2002; Kerth and Morf, 2004), few studies have investigated the distance from which bats can hear these low frequency social calls due to the inherent difficulties in obtaining this information. However, a recent study by Hoffman *et al.* (2007) on the distance from which Greater sac-winged bats *Saccopteryx bilineata* can detect the echolocation calls of conspecifics, has reported an estimated maximum detection distance of 38 metres for echolocation calls broadcast within the forest under the sound transmission conditions of their study site and for an assumed

signal detection threshold of 20 dB SPL (50 m for a threshold of 0 dB SPL) (Hoffmann et al., 2007).

A study on the hearing capacity of *P. auritus* by (Coles et al., 1989) recorded a neural audiogram from the inferior colliculus and found that the *P. auritus* has good auditory sensitivity (less than 10 dB SPL) in the range of 7 – 55 kHz, containing a region of extremely low thresholds between 8 – 20kHz. The most sensitive frequency reached a threshold of -23 dB SPL at 11.7kHz (Coles et al., 1989). At this level it was found that there was heightened response to very faint noise stimuli. For example, even a slight movement by the experimenter (such as breathing) would cause the neural units to fire rapidly (Coles et al., 1989). In the field and when flying, *P. auritus* occasionally uses a loud long-sweep call with a very shallow terminal FM, containing a prominent energy peak at 12kHz (Ahlen, 1981). This frequency corresponds with the most sensitive region reported by Coles et al. (1989) and was reported by both authors to be optimal for long distance communication in the field. However, no published data on the distance from which *P. auritus* detects these low frequency calls has been reported.

Due to the low frequency nature of the simulated *P. auritus* social calls used to elicit responses of radio-tagged *P. auritus*, (as described in Chapter 4) it would be expected that these calls would travel a considerable distance. Therefore, the objective of this experiment was to ascertain the maximum distance from which the simulated social calls would be detected by *P. auritus* under natural conditions (i.e. cluttered woodland environments).

6.2.3.2 – Methods

Eleven *P. auritus* bats (five ♂ and six ♀) were obtained from local bat hospitals. The individuals were long-term captive bats (individuals that could not be released back to the wild due to the nature of their long-term injuries and were kept in captivity for educational purposes). *P. auritus*, in general, can be maintained as captive species relatively successfully (Racey, 1970). The bats were kept individually in mesh cages (Exo Terra Flexarium's 143 Litre capacity measuring 80cm x 40cm x 40cm) for a period of three days. A cotton towel was placed on the inside and allowed to hang up to create a dark compartment for sleeping. Live mealworms (*Tenebrio* spp.) and water were provided. This allowed time for the bats to habituate to their new environment prior to carrying out the experiment and to monitor the behaviour of the individuals. Animal husbandry guidelines for maintenance of the captive individuals were adhered to, as recommended by (Wilson, 1988).

To test the responses of the bats to the Autobat stimulus, each bat was placed initially in the mesh cage inside a large (open-ended) barn. This was to test whether there was an initial response from each bat prior to carrying out the experiment outdoors. The speakers were placed 10 metres back from the Autobat and four synthesised calls were played for a period of one minute with a one minute period of silence in between each call. This was repeated for each of the 11 bats. The behaviour of the bats was noted by the observers and their vocalisations were recorded using a custom-built broadband automated ultrasound recorder.

Two of the 11 captive bats showed no apparent positive response to the calls, both responding by going into torpor and exhibiting signs of stress. These two individuals were returned to the bat hospital and not included in any further experiments. The nine remaining bats were subsequently taken to Plashett Wood and the experiment to determine the distance from which they optimally responded to the stimulus was subsequently conducted.

The experiment was conducted in daylight hours in broadleaved woodland (to prevent potential vocal responses from other bats, which would be active at night-time, confounding the results). The mesh cage with the focal bat was positioned in a sheltered location and natural light was prevented from entering the mesh cage by placing a heavy duty cotton towel on top of the mesh cage. A Sony Handycam (Model DCR – DVD91E) digital video camera recorder was set up to film the bat's responses. Their vocalisations were recorded using a custom-built broadband automated ultrasound recorder.

A distance of 200 metres from the captive bat was measured using a measuring wheel (Trumeter 5000). The Autobat and speaker were placed at an initial distance of 200 metres and once the bat was observed to be 'settled' in the sleeping compartment (i.e. not moving or vocalising as detailed in Figure 6.1), four synthesised *P. auritus* social calls of differing sound intensity (call A, B, C and D see Chapter 4) were broadcast, for a period of one minute each with a period of silence of one minute in between each call. This process was repeated a further 38 times at five metre intervals until the speakers and Autobat were finally at five metres from the captive bat. The time that call was produced was noted. This process was repeated for each of the nine bats. The recorded film was edited using Sony Picture Package™ and the edited film was analysed and the behavioural responses noted. The ultrasound recordings were transferred to a laptop and saved as WAV files. The sound analysis software Bat Sound – Sound Analysis Version 2.1 (Pettersson Elektronik AB, Sweden) was used for the analysis of ultrasound recordings.

6.2.3.3 – Results

Seven of the nine long - term captive bats responded to the Autobat either physically, vocally or both. Two bats (one male and one female) did not respond to the stimulus and appeared to be in torpor when the experiment had finished. Table 6.4 details the responses of each individual to the stimulus. Three bats (two males and one female) responded by continually flying around the cage, echolocating and producing social calls. Two bats (females) responded by continually flying around the cage echolocating but not producing social calls. Two bats (females) responded by physically moving their head in the direction of the stimulus (but not flying). One of these females produced social calls whilst moving her head and the other female moved her head and echolocated. The mean maximum response distance of the responding individuals was 80 m (Range 55m – 105m; SD 15.81, n = 9) and the mean minimum response distance was 7 m (Range 5m – 20m; SD 5.67, n = 9).

6.2.3.4 – Discussion

The experiment with captive bats revealed that the mean maximum response distance to the stimulus was 80 metres (maximum 105m; minimum 55m). Therefore, the lack of response to the stimulus in the trial experiment (Section 6.2.2) would suggest that the focal individuals may have not heard the stimulus (as the mean estimated distance from the focal bats to the Autobat was 300 metres) and, as a consequence, would not be motivated to respond. In order to avoid this scenario, it was essential that the stimulus was broadcast at a distance from which it would be heard by the target individual. Based on the radio-tracking study, experiment and captive bat response estimates, a maximum distance from the stimulus location to the bat, at the start of the experiment, should be no greater than 50 metres.

6.2.3.5 - Conclusions

Captive bats responded to the stimulus in broadleaved woodland at a mean distance of 80m (maximum 105m, minimum 55m). The implementation of a distance of 50m from the focal bat, at each position in the focal bats foraging range (i.e. core, peripheral or exterior), should ensure that the focal bat is close enough to be able to hear the stimulus.

6.3: Responses of females in relation to stimulus location

6.3.1 - Introduction

Once sufficient data had been gathered from each of the 24 females to determine their core and peripheral foraging areas (as detailed in Chapter 5, Section 5.3), an experiment in which synthesised social calls were played at different localities within each female's foraging range was carried out. This section describes the methods and results of this experiment and, also, analyses whether the 24 females followed a predicted pattern of behaviour in relation to the physical response categorisation and the responses to simulated call type, as indicated by the responses of 'non-tagged' bats (see Chapter 4).

6.3.2 – Methods

6.3.2.1 - Locating the focal female

The foraging range for each female was displayed graphically on an OS map. The field workers subsequently approached the focal female on foot and the area in which she currently presented was recorded using GPS and marked onto the map using the "homing-in" method of (White and Garrott, 1990). It was ascertained whether the focal female was in her 'core' (50% KDE), peripheral (areas encompassed within her 95% KDE but excluding areas within the 50% KDE) or if she was in a location previously not recorded. If there was any element of doubt as to her approximate location, or if she was moving rapidly within her range, the field work surveyors waited until she was no longer moving rapidly and her location could be determined more clearly. The starting location (core / peripheral / exterior) for each female was rotated randomly so that previous encounters with the stimulus were unlikely to affect the overall result outcome.

6.3.2.2 - Experimental set up and procedure

Once the starting location for each female was determined, and the focal female was deemed to be within 50m of the stimulus location (a distance we could be confident that focal individual would be likely to hear the stimulus, see Section 6.2.3), the experiment was set up using the equipment and methodology detailed in Section 6.2.2.2. In addition, the Ultrabat, an automated broadband ultrasound recorder, was used to record vocal responses as opposed to the manual recorder used in the pilot study. Figure 6.2 shows an illustration of the experimental set up. If the female had moved significantly during the experimental set up period (approximately 4 minutes), and was considered to be more than 50 meters away from the stimulus, the stimulus location was altered accordingly.

Six different synthesised social calls (both amplified and non-amplified) were produced by the Autobat over a period of one hour (see Chapter 4 Section 4.4.4 for a full description of the calls used). The bats that responded to the Autobat over the one hour period were recorded on video. If the focal female with the radio-tag responded to the Autobat (as opposed to other *P. auritus* individuals in that locality), the LocateABat software registered a response reading indicating that she was within 10 metres at the exact time as the response was recorded on film. After one hour, the experiment was terminated and the process was repeated in the other two range localities. When possible, the experiment for each female was to be completed during one evening. However, in practice, this was not always possible as it was often difficult to locate the bat in peripheral locations during the tracking period (as they visited these areas less often). Therefore, the experiment for some females was conducted over a two or three night period.

Simultaneous bearings for each focal female were recorded for one hour prior to the experiment starting (before stimulus broadcast) and, also, for a period of one hour after the experiment was terminated, in order to detect the focal female movement patterns to assess how, or if, the production of synthesised social calls altered the focal females' behaviour.

6.3.2.3 - Statistical analysis

A chi-square test was used to determine whether there was an association between stimulus location and the number of females responding. Scheirer-Ray-Hare tests were used to examine whether there were seasonal effects on both observed behaviour and simulated call type, or range location effects on both observed behaviour and simulated call type. The observed mean response rate per hour for radio-tagged females, in each behaviour category

and for stimulus call type, in relation to season (i.e. month) was tested for trends using a Jonckheere Terpstra trend test. The responses for each call type were ordered in a manner such that the expected the medians of the group were predicted to change (using the data from the 'non-tagged' bats seasonal responses, chapter 4). So, for example, the order of the mean response per hour for call A of radio-tagged females in each month was compared to the order of the mean response per hour for call A of 'non tagged' bats in each month, in order to assess whether the medians of the groups ascend or descend in the same order. The estimated distance of the radio-tagged bat from the stimulus location during the experimental period was examined using regression analysis. All statistical analyses were carried out using SPSS 16.0 ©SPSS Inc.

6.3.3 - Results

6.3.3.1 - Responses of females to synthesised social calls in relation to range use

The number of individuals that responded to the stimulus and did not respond to the stimulus at different test locations is shown in Figure 6.3. In total, 20 females responded to the stimulus calls produced by the Autobat in their core foraging area (50% KDE) and four females did not respond. Twelve females responded to the stimulus calls produced by the Autobat in the peripheral foraging area (95% KDE excluding 50% KDE) and 12 females did not respond. Five females responded to the stimulus calls produced by the Autobat outside of their foraging range (exterior to 95% KDE) and 19 did not respond to the calls. Table 6.5 details the individuals that responded, the number of responses at each locality, and the females that produced a vocal response. Two females did not respond at all. A chi-sq test

demonstrated that there was a significant association between stimulus location and the number of individuals responding (X^2 = 18.79, d.f. = 2, p < 0.0001).

There was evidence of extensive overlap in the foraging areas of females (investigated further in Chapter 7). This raises the possibility that some individuals may have been preconditioned to the stimulus when it was played for a female whose range overlapped with their own. When data for these eight potentially 'pre-conditioned' females were excluded from the analysis, a subsequent chi-sq test demonstrated that there was still a significant association between stimulus location and the number of individuals responding (X^2 = 12.69, d.f. = 2, p < 0.0017).

Of the 20 females that responded to the stimulus in their core foraging area, 14 responded on more than one occasion (mean = 2.2 responses, SD = 1.15). Of the 12 females that responded to the stimulus in the peripheral foraging area, three responded on more than one occasion (mean = 1.5 responses, SD = 1.16), shown in Figure 6.4. Of the five females that responded to the stimulus outside of their foraging ranges, only one responded on more than one occasion (mean = 1.2 responses, SD = .44). The mean number of times a responding female approached the stimulus in the core foraging area was significantly greater than the mean number of times of approach in the peripheral foraging area or external to the foraging areas (Kruskal-Wallis ANOVA H = 7.466, n = 37, p < 0.024). There was no evidence of either a seasonal effect in the number of responses to the stimulus F (1, 4) = 1.156, p = 0.340 or evidence of an interaction between response in relation to season and range F (1, 8) = 0.504, p = 0.848.

6.3.3.2 - Vocal responses of radio-tagged females to the stimulus

Of the 20 females that responded to the stimulus in their core foraging areas, a vocal response (i.e. social call) was recorded for only nine responding females. A vocal response was recorded for six of the 12 females in the peripheral foraging area and in the exterior foraging area a vocal response was recorded for two of the five responding females. There was no significant difference between the numbers of individuals responding vocally in the core, peripheral or exterior foraging ranges (Kruskal-Wallis ANOVA $H = .154$, $n = 37$, $p < 0.926$).

6.3.3.3 - Behavioural responses of radio-tagged females responding to the stimulus

A total of 81 hours producing synthesised calls resulted in a total of 68 responses to the stimulus from 22 of the 24 radio-tagged females. The 68 responses could be categorised into six of the seven behaviour categories (categories one to six as detailed in Figure 4.16, Chapter 4). There was a significant difference in the mean response rate per hour between categories (Kruskal-Wallis ANOVA $H = 17.818$, $df = 6$, $p = 0.0007$) as shown in Figure 6.5. However, when behaviour seven (unclassified behaviour) was excluded from the analysis no significant difference was found (Kruskal-Wallis ANOVA $H = 7.378$, $df = 5$, $p = 0.194$). Excluding behaviour seven, a Scheirer-Ray-Hare test found that there was also no evidence of a significant difference in response rate per hour between month, behavioural response types or evidence of a significant interaction between month and behavioural response type (test statistics detailed in Table 6.6).

A Scheirer-Ray-Hare test found that there was a significant difference in the mean response rate per hour for radio-tagged females at each range location (i.e. core, peripheral, exterior) but no significant differences in behavioural response types or evidence of an interaction between range location and behavioural response (test statistics detailed in Table 6.7). There were significantly more behavioural responses in the core range compared to the exterior

(Mann U Whitney Z = - 4.059, n = 287, p > 0.0001) and significantly more behavioural responses in the peripheral range compared to the exterior (Mann U Whitney Z = - 2.379, n = 288, p = 0.17), but no significant difference in the number of behavioural responses between the core and the peripheral ranges (Mann U Whitney Z = - 1.907, n = 8289, p = 0.056).

6.3.3.4 – Behavioural responses of radio-tagged females to synthesised social calls (stimuli) of varying intensity.

There was a significant difference in the effectiveness of the call types in eliciting the 68 responses from radio-tagged females (Kruskal-Wallis ANOVA H = 17.818, df = 6, p = 0.022; as shown in Figure 6.6). When the responses from when the stimulus was silent are removed from the analysis, there was still a significant difference in the effectiveness of the call types (Kruskal-Wallis $_{ANOVA}$ H = 11.745, df = 5, p = 0.038). Table 6.8 details the post hoc analysis (using Mann U Whitney with a Bonferroni correction to reduce Type 1 error rate (.05/7) resulting in a critical value for significance of 0.007) to investigate where the differences between call types were significant. Call A was significantly more effective at eliciting behavioural responses to the stimulus compared to call B, but no other significant differences were found in the effectiveness of calls for radio-tagged females (test statistics for pair-wise comparisons detailed in Table 6.8).

A Scheirer-Ray-Hare test found no significant difference between month, call types, or a significant interaction between month and call type for radio-tagged females (test statistics detailed in Table 6.9). There was a significant difference in the mean response rate per hour of radio-tagged females at each range location (i.e. core, peripheral, exterior, as previously

detailed), but no significant differences in call type or evidence of an interaction between call type and range location (test statistics detailed in Table 6.10).

A Jonckheere's trend test was carried out to investigate whether there was a trend in the responses of the radio-tagged females in relation to the observed behavioural responses, using the order of the response categories from the data shown by all non–tagged responding *P. auritus* as a predictor for seasonal response (as detailed in Chapter 4). There was no evidence of a significant trend in the response categories for each month for the radio-tagged females (test statistics detailed in Table 6.11).

A Jonckheere's trend test was also carried out to investigate whether there was a seasonal trend in the response rate of tagged females to each call type that agreed with that found in non-tagged females (as detailed in Chapter 4). Figure 6.7 shows the mean response rate per hour of radio-tagged females to each call type in each month. The only calls that radio-tagged females responded to in May and June were calls A & D (high intensity high repetition) but there was no overall significance in the trend (test statistics detailed in Table 6.12). This is likely to be as a result of the large variation in the response rate per hour (indicated by the large error bars). However, there was a significant trend for Call C (low intensity high repetition), where responses to Call C were only observed for radio-tagged bats in August and September. There also appears to be a pattern whereby high intensity calls elicit responses earlier in the season (May – July), whereas, later in the season, all call types seem equally effective.

6.3.3.5 - Time of response

Each time the stimulus was broadcast, regardless of location in relation to foraging area, the focal female was always within 50 metres of the stimulus. Most responses of radio-tagged females occurred within the first fifteen minutes of the stimulus broadcast, as shown in Figure 6.8, and the number of responses to the stimulus was negatively correlated with experimental time (Spearman rank correlation $r_s = -0.936$, $n = 12$ $p < 0.01$; Figure 6.8). The time of the first response in relation to stimulus location for radio-tagged females was also negatively correlated with stimulus broadcast time in the core foraging areas (Spearman rank correlation $r_s = -0.930$, $n = 12$, $p < 0.01$), negatively correlated (although weaker) with time of first response in the peripheral areas (Spearman rank correlation $r_s = -0.746$, $n = 12$, $p < 0.01$) and, also, outside the foraging area (Spearman rank correlation $r_s = -0.815$, $n = 12$, $p < 0.01$), as shown in Figure 6.9.

6.3.3.6 - Movement of females in their home range in relation to stimulus location

Estimates of location for each female were obtained when the stimulus was broadcast at each range location and for a one hour period after the stimulus had finished broadcasting. For the 22 females that responded, there appeared overall to be a general pattern that, once a female had approached the stimulus (presumably to investigate the source of the sound), her subsequent foraging behaviour was often at a distance of over 150 metres from the stimulus. Once the production of simulated social calls was terminated, the females returned to forage in closer proximity to the stimulus location, as shown for (Bleb 8), in Figure 6.10.

The number of fixes available for females for the hour after their experimental period varied between individuals, because it was not always possible to continue radio-tracking at fixed intervals at the same time as relocating equipment for the next experimental session.

However, a comparison of the time spent at each range location was analysed for each of the 20 females that responded to the stimulus in their core foraging area when the stimulus was broadcasting compared to when the stimulus had terminated broadcasting. Each estimated location of the focal female obtained during the experiment time (a one hour period) and after the experiment was finished (also for a one hour period) was assigned to be within the core, peripheral or exterior range). The proportion of fixes in each locality was converted to a percentage, so if ten fixes were recorded for a female, eight were in the core, two in the peripheral and none recorded outside the range, the proportions would be 80% core, 20% peripheral, and 0% outside known range. The females that responded to the simulated social calls in their core foraging range spent less time within their core foraging range when the Autobat was broadcasting compared to the time spent in the core foraging area when the Autobat had terminated broadcasting (Wilcoxon Signed Ranks $Z = -3.379$, $n = 20$, $p < 0.001$). There was no similar trend found for the females that responded in the peripheral foraging area (Wilcoxon Signed Ranks $Z = -.472$, $n = 12$, $p < 0.637$) or outside the foraging area (Wilcoxon Signed Ranks $Z = -.135$, $n = 5$, $p < 0.892$).

6.3.4 – Discussion

The main aim of the study was to investigate the response of female *P. auritus* to simulated social calls in relation to range use. Using the results of the radio-tracking data, detailed in Chapter 5 Section 5.2, the stimulus location was set up to within 50 metres of the focal female, in areas deemed to be within her core and peripheral foraging range and, also in an area outside of her range (where she had previously not been recorded in the radio-tracking study).

Females were more likely to respond to simulated social calls played in their core foraging range than in their peripheral foraging range or outside of their range. To date, no other studies have been carried out on bats where playback (or the use of synthesisers to produce simulated calls) has been used as a tool to investigate the responsiveness of individuals to the stimulus in relation to range use. However, similar studies have been undertaken with other animals. For example, a study on a wild population of Swift foxes *Vulpes velox* found that male territory holders responded more intensely with barking if a playback simulating intrusion by a rival occurred inside of the core (50% kernel contour isoline of estimated home range) compared to outside (Darden and Dabelsteen, 2008). The authors reported a high degree of overlap in the home range core by neighbouring home ranges (similar to *P. auritus* see Chapter 7) so territoriality exclusivity was unlikely to account for the increased intensity of response. In contrast, a study on Grey wolves *Canis lupus* using playback of simulated wolf howls found that the responses to simulated intrusions were independent of the location within the home range (Harrington and Mech, 1983). The propensity of *P. auritus* to respond maximally to simulated social calls in core foraging areas is consistent with the resource defence hypothesis. Repelling intruders from core foraging areas may reduce competition for resources within them.

Resource defence has been suggested as the underlying cause of aggressive interactions, characterised by behaviour such as chases and low frequency vocalisations, in feeding areas for species such as the hoary bat *Lasiurus cinerus* (Belwood, 1982; Fullard, 1982) and pipistrelle bats (Barlow and Jones, 1997) which, in turn, appeared to be associated with the abundance of prey (i.e. aggressive behaviours and social call production increase at low insect densities). For other bat species studied in the field in considerable detail, such as

Myotis lucifigus, (Fenton et al., 1976; Anthony and Kunz, 1977; Fenton and Bell, 1979; Aldridge et al., 1990), there has been no reported evidence of aggressive encounters between foraging individuals. However, these are species that use multiple feeding areas and not small areas of woodland like *P. auritus*.

Resources may also only be worth defending at particular times of year (e.g. birth and lactation when energy demands are greatest) or against particular types of individuals within the population (i.e. roost members versus strangers). In this study, however, there was no evidence of a significant difference in the number of responses for radio-tagged females in relation to season. Nonetheless, the time of first response to the stimulus, for the majority of females, tended to be prompt (i.e. most responses occurred within 15 minutes of the stimulus broadcast), indicating that perhaps the inclination to respond to the stimulus may be as a necessity to investigate unfamiliar ultrasound, which resemble intra-specific social calls, within their foraging range.

There was no evidence of a significant seasonal effect in call type for eliciting responses from radio-tagged females. However, only four females were tested in May and June, and all of their responses were to high intensity high repetition calls. In July, five females were tested and all of their responses were also to high intensity calls with both high and low repetition, whereas in August and September there was evidence of responsiveness to all call types. In heart-nosed bats *Cardioderma cor*, (Vaughan, 1976) reported a loud audible call with energy at 12kHz which is used to establish exclusive foraging areas. It may be that, earlier in the season, the propensity of *P. auritus* to respond maximally to loud high intensity calls indicates that these call types are used to establish vital foraging areas, close to roost sites, to

ensure successful reproduction at a critical time of year. There was also a significant trend in response to low intensity high repetition calls (such as call C) later in the season. The function of such a call type may could, for example, be related to mating or mate attraction. However, it would be necessary to test the responses of both males and females to confirm such a prediction. It could easily be that the function of different call types changes throughout the year, as demonstrated for various species of birds (Timcke and Bergmann, 1994; Lucas et al., 2007).

An alternative explanation to the resource defence hypothesis could be that the response to stimulus may represent attraction to resources. Responses to calls could represent co-ordination of foraging (i.e. attraction to food resources). Greater spear-nosed bats, *Phyllostomus hastatus*, are known to emit audible screech calls which appear to function as contact calls that recruit and co-ordinate foraging among group members (Wilkinson and Boughman, 1998). Food associated calls have also been demonstrated for other species, particularly primate species (Gros-Louis, 2004; Gros-Louis, 2006; Clay and Zuberbuhler, 2009; Slocombe et al., 2010). Advertising food sources to conspecifics may be beneficial to maintain a stable social group if food sources are in abundance (i.e. the benefits of sharing outweigh the costs). If *P. auritus* females response to the stimulus represents attraction to resources, then it would be expected that there would be some evidence of co-ordination of foraging between females. This is investigated further in Chapter 7.

Finally, on a practical note, the declining responsiveness of radio-tagged females may represent habituation to the stimulus over time, reducing its effectiveness for use as a lure. Once a radio-tagged female responded to the stimulus in her core foraging area, there was a tendency to move away from the stimulus to forage elsewhere whilst the stimulus continued

to broadcast. There was evidence that females spent less time in their core foraging areas when the stimulus was broadcasting for 60 minutes compared to the subsequent 60 minutes when the stimulus finished broadcasting. Therefore, there is the potential for the stimulus to alter female foraging behaviour, which, if used continuously at foraging sites, could affect the fitness of *P. auritus* females by deterring them from core feeding areas.

6.4 – Summary

- The experiment showed that female *Plecotus auritus* were significantly more likely to respond to a stimulus produced within their core foraging area, than in peripheral area or outside their foraging area.
- Female *Plecotus auritus* also approached the stimulus significantly more often in the core foraging area compared to the peripheral foraging area and external to the foraging area.
- There were no significant differences in behavioural response types and no evidence of an interaction between range location and behavioural response.
- The time of first response in relation to stimulus location for radio-tagged females was negatively correlated with stimulus broadcast time.

	Cluttered habitat field test 1			Cluttered habitat field test 2			Reliability Test - Retest	Reliability Test - Retest
Distance	**Mean open field reading (volts)**	**SD**	**LocateABat Output**	**Mean woodland reading (volts)**	**SD**	**LocateABat Output**	**Pearson Correlation**	**Significance**
2	4.5835	0.03014	1000 - 990	4.5815	0.02834	1000 - 990	0.474	**0.035**
5	2.8775	0.03401	989 – 927	2.8370	0.11563	989 – 927	0.523	**0.018**
10	1.8665	0.05566	926 – 642	1.8990	0.02594	926 – 642	0.453	**0.045**
15	0.9295	0.07156	641 - 501	0.9700	0.06087	641 - 501	-0.092	0.701
20	0.5895	0.14409	n/a	0.6640	0.21303	n/a	-0.287	0.220
25	0.4615	0.44683	n/a	0.3510	0.14160	n/a	-0.150	0.528

Table 6.1: LocateABat trial experiment results to test for accuracy of the system in cluttered environments. The volts reading indicate the reading on a voltmeter used in the field. The 'LocateABat' output details the range readings obtained from the system at differing distance intervals. The Pearson correlation coefficient for the total test-retest score of both tests at each measurement interval (and the significance of the results) is detailed.

	Open field test 1			Open field test 2			Reliability Test - Retest	Reliability Test - Retest
Distance	**Mean open field reading (volts)**	**SD**	**LocateABat Output**	**Mean woodland reading (volts)**	**SD**	**LocateABat Output**	**Pearson Correlation**	**Significance**
2	4.5880	0.03302	1000 - 990	4.5945	0.02665	1000 - 990	0.866	**0.010**
5	2.9350	0.03517	989 – 927	2.9425	0.04266	989 – 927	0.714	**0.010**
10	1.8710	0.04994	926 – 642	1.8945	0.03940	926 – 642	0.546	**0.013**
15	0.9375	0.05571	641 - 501	0.9420	0.06950	641 - 501	0.560	**0.010**
20	0.6110	0.12945	n/a	0.6905	0.21765	n/a	-0.115	0.631
25	0.3535	0.11860	n/a	0.3535	0.16096	n/a	0.367	0.112

Table 6.2: LocateABat trial experiment results to test for accuracy of the system in open environments. The volts reading indicate the reading on a voltmeter used in the field. The LocateABat output details the range readings obtained from the system at differing distance intervals. The Pearson correlation coefficient for the total test re-test score of both tests at each measurement interval (and the significance of the results) is detailed.

40% HM Core						**80%HM Peripheral**					**Exterior**				
Bat	Response	Number of responses	Estimate of how close bat approached (m)	Mean distance from stimulus	SD	Response	Number of responses	Estimate of how close bat approached (m)	Mean distance from stimulus	SD	Response	Number of responses	Estimate of how close bat approached (m)	Mean distance from stimulus	SD
2♀	Yes	2	1	55	41	No	n/a	240	281	36	No	n/a	364	448	94
3♀	Yes	1	2	113	75	No	n/a	129	185	34	No	n/a	183	299	92
4♀	Yes	3	1	92	123	Yes	1	2	185	138	No	n/a	213	270	33
5♂	No	0	55	251	142	No	n/a	93	261	246	No	n/a	287	303	14
6♀	Yes	3	1	31	26	No	n/a	39	65	18	No	n/a	318	402	71
7♀	Yes	2	1	42	39	Yes	1	1	73	43	Yes	1	2	83	49

Table 6.3: Response of radio-tagged *P. auritus* to Autobat and estimates of distances (based on triangulation) from stimulus, during playback in 40% HM (core), 80% (peripheral) and outside (exterior to MCP) foraging range.

Figure 6.1: *P. auritus* prior to the stimulus being played. The bat is settled in the sleeping compartment.

Bat no.	Sex	Date of expt.	Response to stimulus	Maximum response distance (m)	Minimum Response distance	Flight	Attentive	Social Calls	Echolocation
3	Male	03.5.07	Yes	90	5	✓	✓	✓	✓
4	Male	03.5.07	No	n/a	n/a	×	×	×	×
5	Male	05.5.07	Yes	75	5	✓	✓	✓	✓
6	Female	05.5.07	Yes	80	5	✓	✓	×	✓
7	Female	14.5.07	Yes	105	5	✓	✓	✓	✓
8	Female	14.5.07	No	n/a	n/a	×	×	×	×
9	Female	14.5.07	Yes	55	5	×	✓	✓	✓
10	Female	20.5.07	Yes	85	5	×	✓	×	✓
11	Female	20.5.07	Yes	70	20	✓	✓	×	✓

Table 6.4: Responses of captive *P. auritus* to simulated social calls, produced by the Autobat, in broadleaved woodland. The maximum and minimum distance each individual responded, along with the behaviour for each bat is detailed.

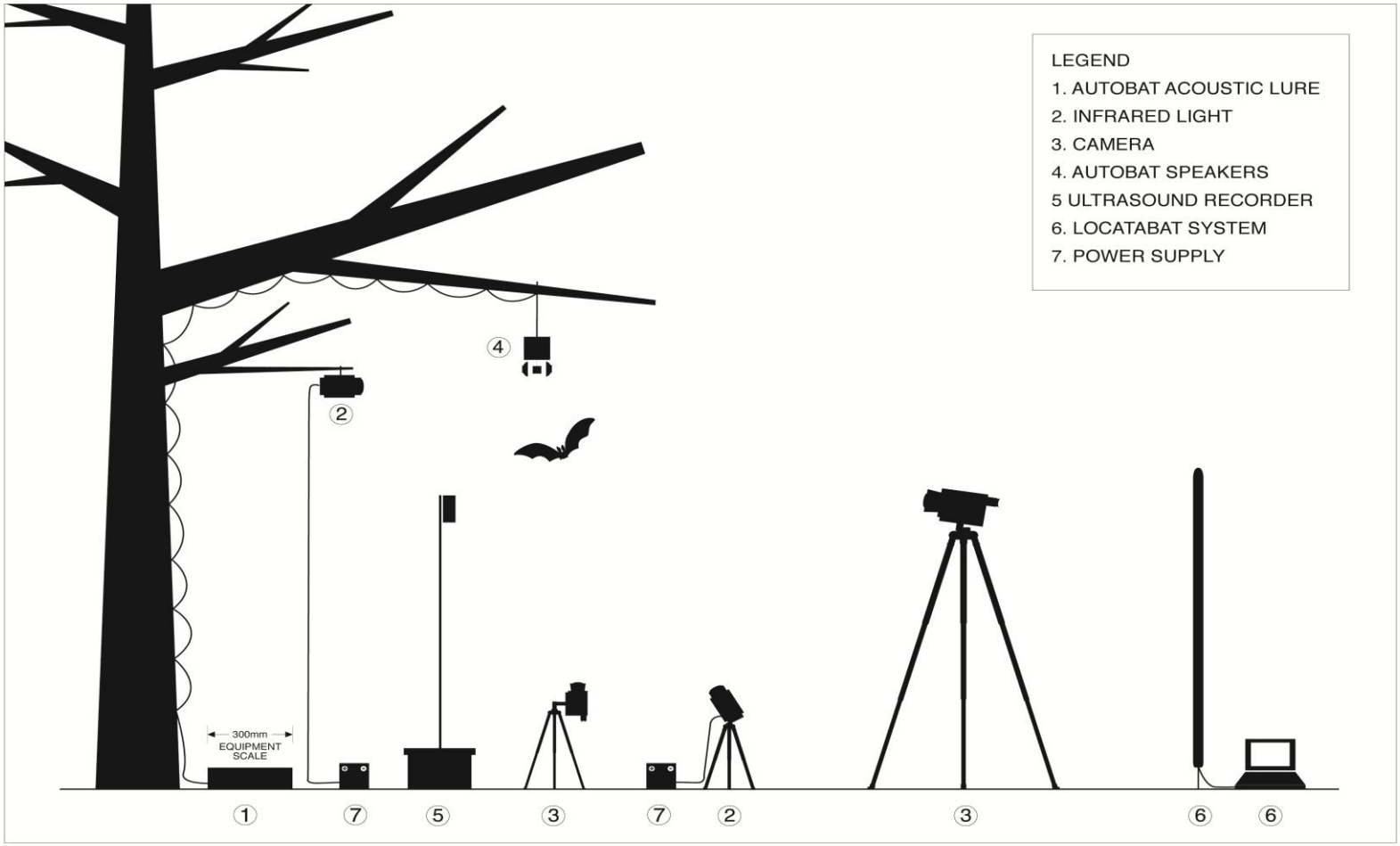


Figure 6.2: Positions of the equipment used in the LocateABat experiment to playback synthesised *P.auritus* social calls to radio-tagged females to assess their response in relation to range use.

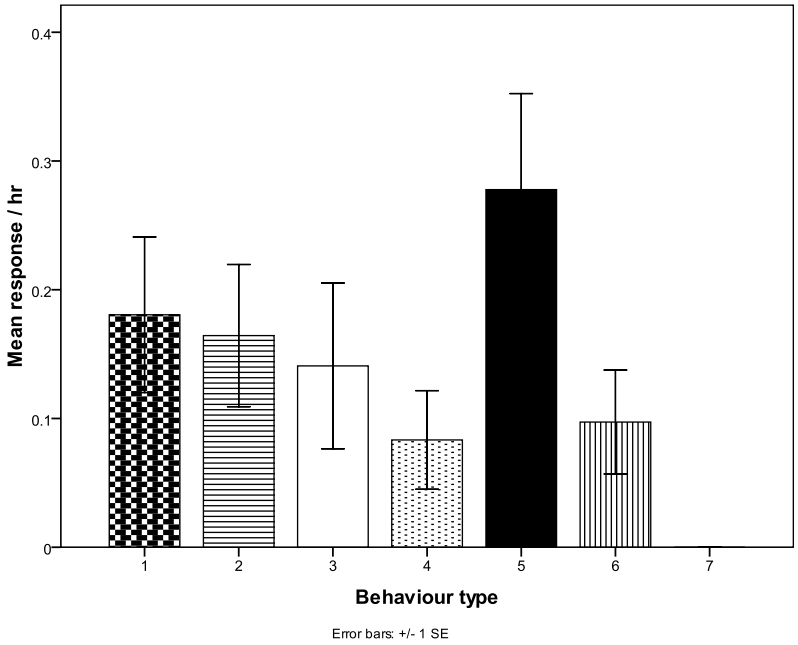


Figure 6.5: The mean response rate / hr of the 24 radio-tagged females to each behavioural category. There was a significant difference in the mean response rate per hour between categories (Kruskal-Wallis ANOVA H = 17.818, df = 6, p = 0.0007). However, when category 7 (unclassified behaviours) was excluded no significant difference was found in the mean response rate per hour between categories (Kruskal-Wallis ANOVA H = 7.378, df = 5, p = 0.194).

Source of variation	**SS**	**SS/MS total**	**d.f.**	***P* - value**
Month	2.629	2.308	4	0.6796
Behaviour response type	0.598	10.151	5	0.0721
Month*Behaviour response type interaction	3.879	14.977	20	0.7782

Table 6.6: The results of the Scheirer–Ray–Hare test showed that there were no significant differences in relation to month, behavioural response type nor a significant interaction between month and behavioural response type.

Source of variation	**SS**	**SS/MS total**	**d.f.**	***P* - value**
Range location	4.593	17.73	2	0.0001
Behaviour response type	1.768	6.82	5	0.2342
Range location *Behaviour response type interaction	2.014	7.77	10	0.6513

Table 6.7: The results of the Scheirer–Ray–Hare test showed that there were significant differences in the number of responses in relation to range location, but no significant differences in behavioural response type nor evidence of an interaction between range location and behavioural response type.

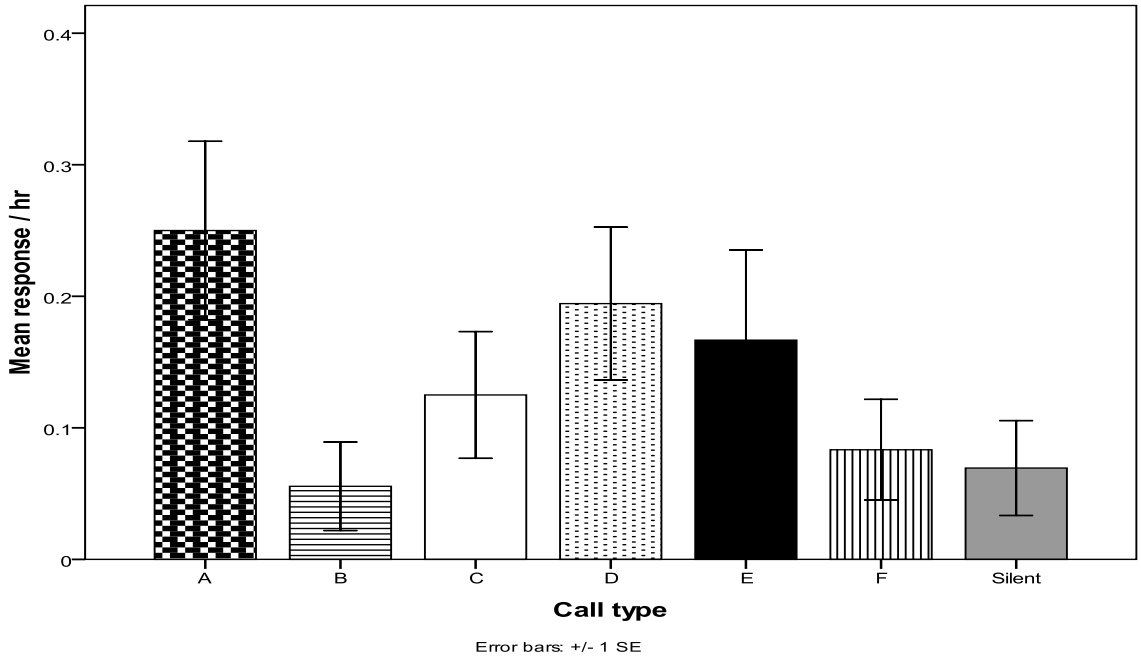


Figure 6.6: Mean number of responses per hour, of radio-tagged females, to each call type (Kruskal-Wallis ANOVA H = 17.818, df = 6, p = 0.022).

Call type	A	B	C	D	E	F	Silent
A		**Z = - 2.813, n = 144, p = 0.005**	Z = - 1.627, n = 144, p = 0.454	Z = - 0.490, n = 144, p = 0.624	Z = - 1.362, n = 144, p = 0.173	Z = - 2.211, n = 144, p = 0.027	Z = - 2.504, n = 144, p = 0.012
B			Z = - 1.301, n = 144, p = 0.193	Z = - 2.415, n = 144, p = 0.016	Z = - 1.558, n = 144, p = 0.119	Z = - 0.715, n = 144, p = 0.475	Z = - 0.381, n = 144, p = 0.703
C				Z = - 1.174, n = 144, p = 0.240	Z = - 0.272, n = 144, p = 0.786	Z = - 0.613, n = 144, p = 0.540	Z = - 0.942, n = 144, p = 0.346
D					Z = - 0.906, n = 144, p = 0.365	Z = - 1.779, n = 144, p = 0.075	Z = - 2.087, n = 144, p = 0.037
E						Z = - 0.881, n = 144, p = 0.378	Z = - 1.205, n = 144, p = 0.228
F							Z = - 0.338, n = 144, p = 0.735

Table 6.8: Pairwise comparison between different response rates for each call type. Using a critical value of 0.007, the only significant difference in response rates for radio-tagged females to each call type was between call type A and B, where call A elicited significantly more responses than call B.

Source of variation	SS	SS/MS total	d.f.	*P* - value
Month	0.570	2.436	4	0.6796
Call type	2.531	10.816	5	0.0552
Month*Call type interaction	4.299	18.372	20	0.5629

Table 6.9: The results of the Scheirer –Ray – Hare test showed that there was no significant differences in relation to month call type or evidence of a significant interaction between month and call type for radio-tagged females.

Source of variation	SS	SS/MS total	d.f.	*P* - value
Range location	3.868	16.53	4	0.6796
Call type	1.892	8.080	5	0.0552
Range location*Call type interaction	1.927	8.234	20	0.5629

Table 6.10: The results of the Scheirer –Ray – Hare test showed that there was a significant difference in the mean response rate per hour in relation to range location but no significance difference in relation to call type or evidence of a significant interaction between call type and range location.

Behaviour Response category	J-T – Statistic	df	*P*_value
1	0.978	5	0.3281
2	0.725	5	0.4692
3	1.136	5	0.2854
4	1.521	5	0.1281
5	1.652	5	0.0962
6	1.337	5	0.1813

Table 6.11: The results of the Jonckeere's trend test indicated that the radio-tagged female responses did not follow a predicted pattern in relation to season (using the non-tagged bats responses as the specified order of response).

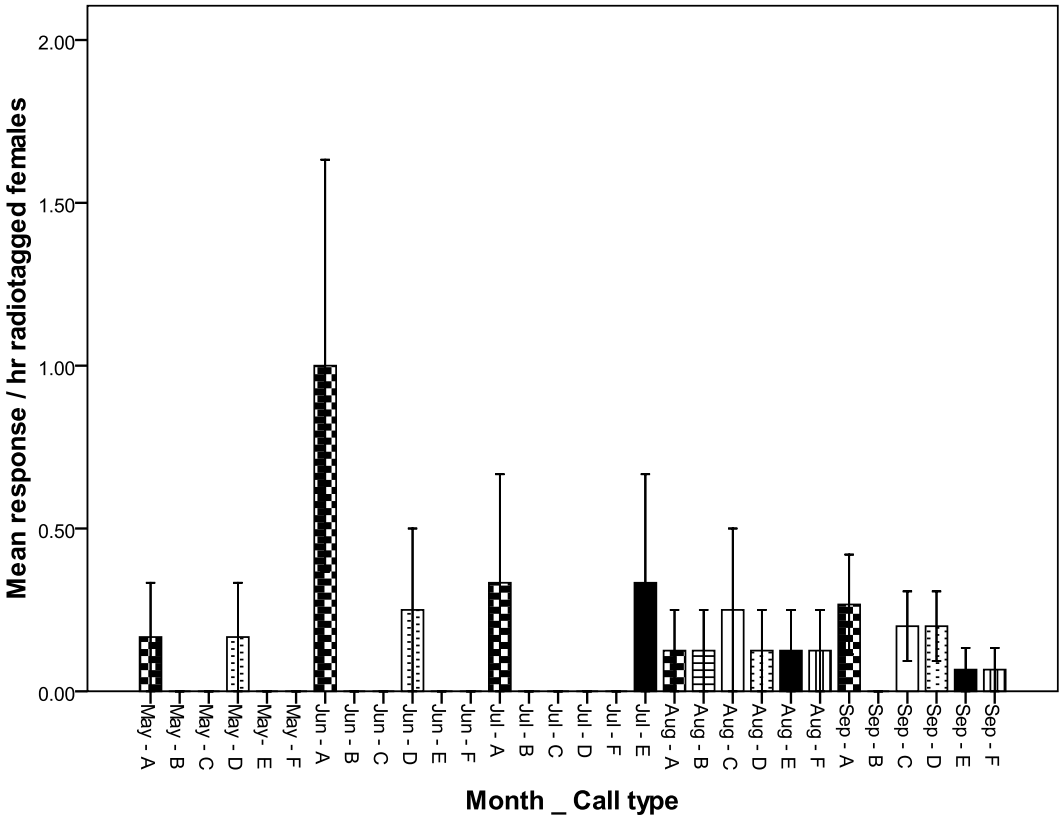


Figure 6.7: Mean response rate per hour for each call type in each month for radio-tagged females.

Call Type	J-T – Statistic	df	*P*_value
A	1.395	5	0.1632
B	0.425	5	0.6708
C	3.126	5	0.0018
D	0.971	5	0.3315
E	0.232	5	0.8165
F	0.545	5	0.5858

Table 6.12: The results of the Jonckeere's trend test indicated that the radio-tagged female responses followed a predicted pattern in relation to season (using the non-tagged bats responses as the specified order of response) for call C only.

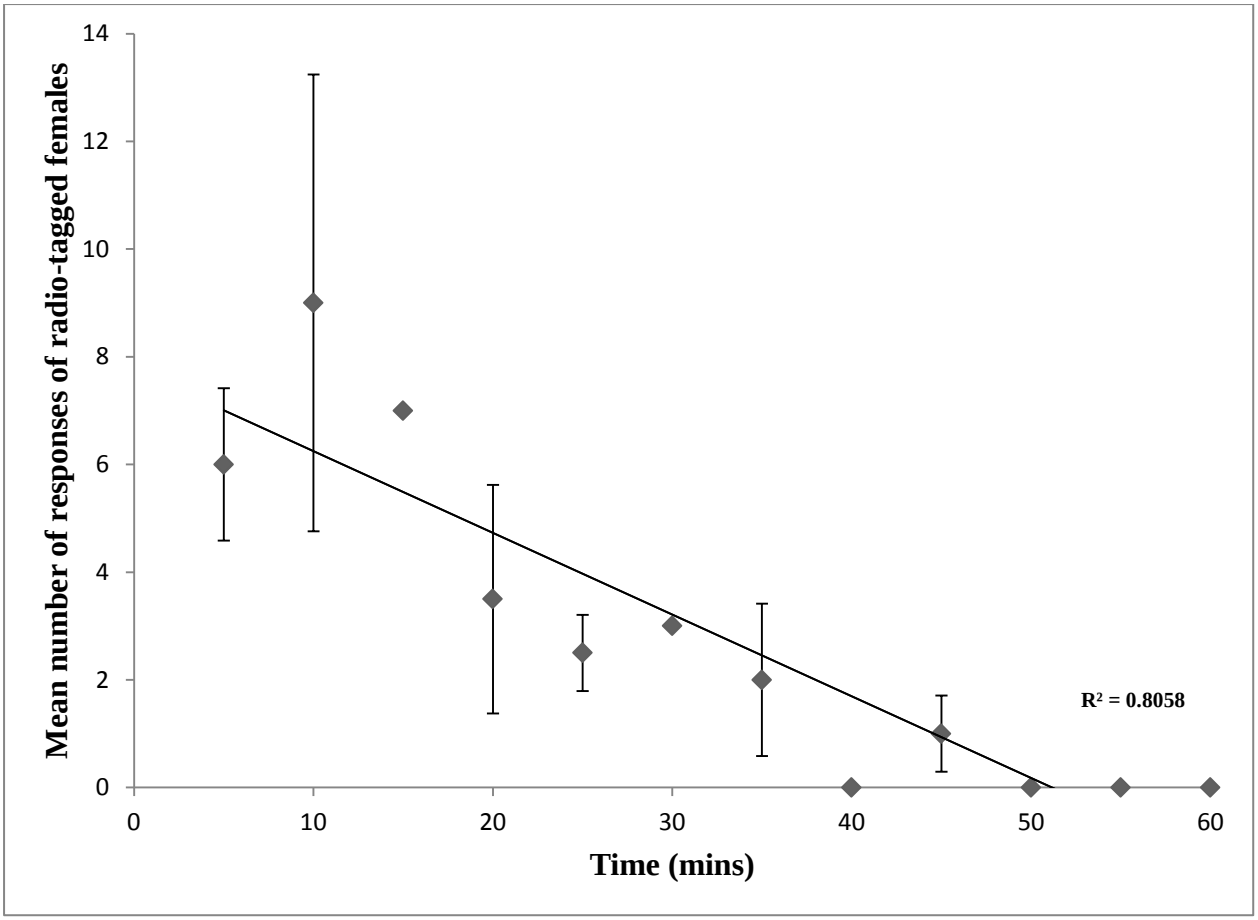


Figure 6.8: Mean number of responses to synthesised social calls broadcast over a 60 minute experimental period. The error bars show the standard deviation between years (i.e. means in 2007 and 2008). The mean number of responses was negatively correlated with experimental play time, i.e. most radio-tagged females responded within the first 15 – 20 minutes of the stimulus broadcast).

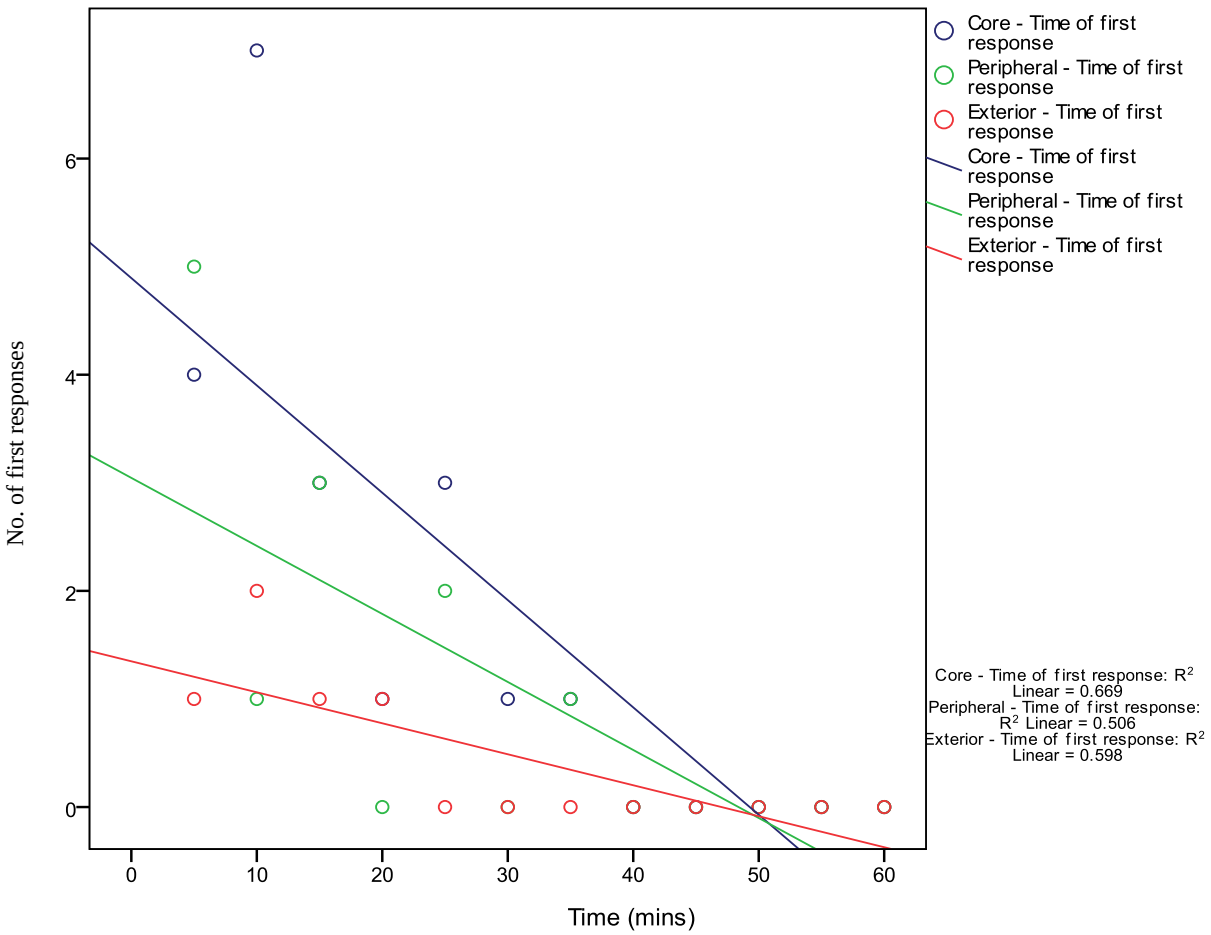


Figure 6.9: The number of first responses to simulated *P. auritus* social calls by radio-tagged *P.auritus* in relation to their core, peripheral and exterior foraging localities. Most responses occurred within the first 30 minutes and the time of first response was negatively correlated with time at all localities.

7.1 - Introduction

There was evidence of spatial overlap in home range use between female *P. auritus* radio-tracked at a variety of sites (Chapter 5). The amount of overlap in individual home ranges can provide indirect information about the likelihood of social interactions (Shier and Randall, 2004; Chaverri et al., 2007; Harless et al., 2009) and has been described as an index of 'static interaction'(Macdonald et al., 1980). However, such an index is of limited value because it does not take into account the utilisation distribution of shared parts of the range (Doncaster, 1990). For example, two females may have an area of overlap in their foraging ranges but seldom meet there because most of their foraging activities are concentrated in non-overlapped areas, or they use the overlapping areas at different times. On the other hand, two females with a similar area of overlap may concentrate their foraging activities on that common area.

Simultaneous radio-tracking of two or more individuals allows each animal's movements to be analysed in relation to the movements of other individuals. This analysis, termed 'dynamic interaction', uses the simultaneous locations to assess temporal aspects of interaction within the context of home range use (Doncaster, 1990; Minta, 1992). Dynamic interaction analysis has been used in a variety of radio-tracking studies to investigate the interactions of animals such as badgers (Bohm et al., 2008), foxes (White and Harris, 1994), and coyotes (Chamberlain et al., 2000). In these studies, interactions are defined as two individuals being recorded within a critical distance of one another which, in turn, is determined by the radio-tracking regime.

The overlap of home range means that there is the potential for females to coordinate their foraging activities. Co-ordinating foraging through passive information transfer has been proposed as a reason for group formation of male bats in species foraging for ephemeral insects in temperate zones (Safi and Kerth, 2007). More active information transfer has been demonstrated by Wilkinson and Boughman (1998) in greater spear-nosed bats *Phyllostomus hastatus*. In this species screech calls appear to act as contact calls that recruit other members of the colony to rich food resources, thereby coordinating foraging among group members. If *P. auritus* females overlap considerably in home range, and show some evidence of co-ordinating foraging, then the responses to the Autobat stimulus calls could represent attraction to what they perceive as recruiting calls, in which case the resource defence hypothesis may not account for female propensity to respond.

This study aimed to: (i) estimate the foraging range overlap of simultaneously radio-tracked dyads caught at the same location; (ii) assess the extent to which roosting associations and overlap of foraging areas are related (iiii) examine the interactions between simultaneously radio-tracked *P. auritus* females in relation to their movements; (iv) determine the extent of range and core area overlap of non-simultaneously radio-tracked females radio-tracked at the same site within the same year. If responses to the Autobat stimulus represent approaches to what are perceived as recruitment calls, then it would be expected for female ranges to overlap considerably and show some evidence of co-ordination of foraging between pairs of females that share their range.

7.2 - Methods

7.2.1 - Capture and selection of study animals

Between August 2007 and September 2008 20 females, comprising 10 pairs, were captured at the study sites and fitted with radio-transmitters, as detailed in Chapter 5. Bats were caught in pairs, either in mist nests or harp traps, to which they were attracted using an acoustic lure (Sussex Autobat: Hill & Greenaway, 2005) that produced synthesised social calls (as detailed in Chapter 4). The pairs of females were always caught and radio-tagged on the same night. In order to maximise the chances of catching a pair with adjacent or overlapping foraging areas two capture sites (occasionally three) were located in the woodland interior away from edges and rides at a distance of approximately 50 metres apart. Trapping began approximately 60 minutes after sunset and lasted for approximately 2 hours. If more than two females were caught at the two capture sites, individual females caught in the same trap or net were selected for radio-tagging in preference to females caught in two traps or nets. If more than two females were caught in the same trap then those females caught closer together in time were selected for radio-tagging.

7.2.2 - Radio-tracking methodology and home range analysis

For each pair of females the day roosting location of each individual was recorded over the radio-tracking period and the females were radio-tracked synchronously from emergence to re-entry. Synchronous radio-tracking involved taking bearings from one individual from the dyad followed immediately by taking bearings from the other member of the dyad. Simultaneous radio-tracking of dyads allows for the calculation of separation distances at five minute intervals in order to assess direct interactions and movement patterns with respect to

each other. If one member of the dyad moved too far away from the other to be simultaneously radio-tracked then, if possible, the surveyors would switch between individuals at 15 minute intervals in order for accurate bearings to be taken. Pairs of compass bearings, and the locations they were taken from, were used to estimate the locations of the foraging *P.auritus* dyads. Home range boundaries were defined by 100% MCP and 95% KDE and the core foraging areas were determined by 50% KDE (as detailed in Chapters 5 and 6).

7.2.3 - Overlap analysis

The degree of overlap of simultaneously radio-tracked dyads and, also, of individuals that were radio-tracked, at the same site in the same year (but not simultaneously), was assessed by calculating the proportional overlap of all individual foraging ranges 100% MCP, 95% KDE, 50% KDE using the program Ranges7eXtra v1.8 Anatrack Ltd. Percentage range overlap of each home range estimator was calculated in Ranges using the formula:

$$(Rzy/Rz + Ry) \times 2$$

where *Rzy* represents the size of the region of overlap between bats *z* and *y*, and *Rz* and *Ry* represent the total range size of bats *z* and *y*. By multiplying this number by 2, the average area of overlap within each bat's range was obtained.

An index of range overlap was also calculated for simultaneously radio-tracked individuals. An association index (used also by Chamberlain et al., 2000 which modified the simple ratio used by Ginsberg and Young (1992) to quantify association:

$$n_1 + n_2 / (N_1 + N_2) \times 100$$

where n_1 and n_2 are the number of locations for the bats within the overlap region and N_1 and N_2 are the total number of fixes recorded for each bat. An index of roosting association for each dyad was also calculated by dividing the number of days the females shared roosts by the total number of days their roosting location was recorded. Therefore, if the simultaneously radio-tracked dyad shared the same roost every day the index would give a value of 1. On the other hand, if they did not share a roost during the radio-tracking period, the index would give a value of 0. This index of roosting association was used to investigate whether the degree of foraging range overlap was related to how frequently pairs of females roosted together or not.

7.2.4 - Spatial Association

A grid-based spatial analysis of the estimates of locations for each dyad was applied to test for association using a pair-wise comparison test of association (Schluter, 1984). Spatial Association is a grid-based test that compares the presence or absence of different point patterns in each grid quadrat. Pairwise results are based on a Chi-squared test between all possible pairs of point patterns selected for comparisons. Yates correction factor was applied to account for bias resulting from cases of low cell frequencies, reducing the chi-square value obtained and, thus, increasing its p-value (Armitage and Berry, 1994).

The Jaccard index (also known as the Jaccard similarity coefficient) is a statistic used for comparing the similarity and diversity of sample sets. The Jaccard similarity index considers the similarity between two units as the number of attributes shared divided by the total number of attributes present in either of them (Waite, 2000) and may be expressed as follows:

Jaccard Index: $J(A,B) = |A \cap B| / |A \cup B|$.

The Jaccard similarity index was calculated for each dyad using estimates of location in Biotas 1.03, Ecological Software Solutions Ltd.

7.2.5 - Dynamic interaction analysis

Overlap in range area does not necessarily indicate that the individuals frequently encounter each other because they may rarely visit the same place at the same time (Macdonald et al., 1980). Doncaster (1990) called the analysis of overlapping range 'static interaction' (described above) and proposed the examination of 'dynamic interaction' by looking at estimates of locations taken at the same time. Analysis of dynamic interactions, as described by Doncaster (1990), compares the *n* observed separation distances from simultaneously radio-tracked pairs against baseline data made up of the n^2 artificially paired separation distances from the same sample using χ^2 tests. However, it has been questioned whether the comparison of 'real' separation distances with unpaired separation distances, which bear no resemblance to a biologically meaningful movement path, undermines the validity of the test (White and Harris, 1994). Furthermore, the original publication, which provided a test of whether two individuals showed significant attraction or avoidance, depended on two assumptions: firstly, that locations are statistically independent and secondly, that their distribution fitted a parametric model. It was not possible to assume independence from the distribution of locations between single pairs of females.

To investigate whether paired females were more cohesive than expected by chance, a single statistic for each range was compared using observed and possible distances between animals, as recommended by Kenward et al., (1993). The mean, geometric mean and median distances were estimated between the n observed pairs of same-time locations for animals one and two. The equivalent values were then estimated for the n x n possible distances (if animal two could be at any of its n used positions when animal one was at each of its used positions). The observed and possible distances were subsequently compared using the Jacobs Index (Jacobs, 1974), which gives a value of 0 if the observed and possible distances were the same, rising towards +1 if observed distances were small relative to possible distances (because the animals were usually together) or falling towards -1 if animals tended to avoid each other. The Jacobs index formula is:

$$D = \frac{de - do}{de + do}$$

(where *de* is the expected distance and *do* is the observed distance between pairs of simultaneous locations). This gives a single index for each pair of animals, which tends to be most consistent if based on the geometric mean distances (Walls and Kenward, 2001). The existence of a dynamic interaction does not necessarily imply mutual awareness on the part of the respective animals (Chamberlain et al., 2000), but addresses whether animals are more likely (positive interaction) or less likely (negative interaction) to maintain a certain separation distance than would be expected from the configuration and utilisation of areas within their known home ranges (Doncaster, 1990).

7.3 - Results

7.3.1 - Overlap of simultaneously radio-tracked females

Thirty-six nights of simultaneous radio-tracking were recorded from 10 different dyads. One dyad showed no overlap in any measure of range area. Another dyad showed less than 3% overlap in the 100% MCP, and no overlap in other measures (Pairs three and five, detailed in Appendix A.7.1). The percentage overlap for the remaining eight dyads (16 individuals) varied and ranged from 23.4% to 92.3% (mean 51%; sem = 4.9%) in the 100% MCP; 17.3% to 97.9% (mean 53.5%; sem = 5.8%) in the 95% KDE; and 17.5% to 96.8% (mean 46.8%; sem 6%) in the 50% KDE. There was no significant difference between the mean amounts of overlap, for each female, between the three range estimators (100% MCP, 95% KDE and 50% KDE) (Kruskal-Wallis ANOVA $H = 1.474$, $n = 48$, $p = 0.479$). This variation in the extent of overlap is exemplified by dyads radio-tracked at Capite Wood. Pair three (Bleb 17 and 18), were caught at the same location in the woodland and subsequent radio-tracking data revealed that this pair had no overlap in any of the range estimators employed. However, by comparison, pair eight (Bleb 27 and 28), also caught at the same location had approximately 25% overlap in the 100% MCP and 95% KDE and approximately 20% in the 50% KDE for each individual, as illustrated in Figures 7.1, 7.2 and 7.3.

7.3.2 - Spatial Association

The grid-based spatial association test (Schluter, 1984) was used to compare the presence or absence of different point patterns (estimates of location) in each grid across the range. The

test found that there was a significant positive spatial association for eight of 10 dyads, as detailed in Table 7.1. Two dyads (pair three and pair five) had a negative association. For example, at Capite Wood pair three had no association (Figure 7.4a) as there were no grids that shared estimates of location. Whereas, by comparison, pair eight had a positive association (Figure 7.4b), as they were nine grids sharing estimates of location from both females.

7.3.4 - Range overlap and roosting association of simultaneously radio-tracked P. auritus

For each dyad both females were located in their roosts on between three and six days (Table 7.2). For three pairs the females were always found roosting separately (roosting index = 0), while for three pairs the females were always found in the same roost (roosting index=1). The remaining four pairs were found roosting together on some days and apart on others. There was a significant positive correlation between roosting index and the Jaccard index (Spearman $r^2 = 0.876$, $p<0.0009$), indicating that dyads with greater overlap in their ranges roosted together more often (Figure. 7.5).

7.3.5 - Dynamic interactions of simultaneously radio-tracked dyads

A total of 1044 ‘paired observations’ were obtained from the ten dyads throughout the radio-tracking period. The number of paired observations for each dyad ranged from 37 to 188 (mean 104.4; SD = 50.05). The mean distance between pairs of fixes for each dyad ranged from 50.70 metres to 251.88 metres (mean 131.05; SD = 68.38). The Jacob index value for each dyad ranged from 0.006 – 0.315 (mean = 0.142; SD = 0.130). Table 7.3 shows the results of the dynamic interaction analysis. Six of the ten adult female pairings were close to

zero, indicating neither avoidance nor attraction during foraging. One pair, pair six, lactating females who shared the same roost, did show evidence of a positive interaction (Jacob index = 0.315). Adult – juvenile pairings (n = 3) had a tendency to have a greater interaction when foraging, (Jacob index, 0.230, 0.292, 0.312 respectively), suggesting that adults and juveniles may associate more frequently compared to adult females when foraging (Fishers Exact Probability 2 x 3 p = 0.0333). The distance between paired observations for each dyad was categorised into distance bands of 50 metre intervals. The percentage of observations in each distance category, for each dyad, is shown in Table 7.4.

7.3.6 - Range overlap of all simultaneously and non-simultaneously radio-tracked individuals radio-tracked at the same site within the same year

Overlap was also found in the foraging ranges of females that were not radio-tracked simultaneously (but were radio-tracked within the same year). The proportion of overlap, in the 100% MCP, 95% KDE and 50% KDE, for each female with other females radio-tracked at the same site, in the same year, are detailed in the Appendix (A.7.2, A.7.3 and A.7.4). The amount of overlap did vary between sites. However, a comparison is not possible due to the likelihood that only a small proportion of the bats from each site were radio-tracked. Nonetheless, the overlap between adjacent females at one site, Hoe Wood, was extensive. For example, one female (Bleb 24) range (95% KDE) overlapped by 50% with four other females. Her core area also overlapped extensively (27% to 52%) with those four females. Figure 7.6 shows the overlap in the core foraging area of six females radio-tracked at Hoe the 14th July 2008 and the 11th August and 2008. Overlap in the core area for females at this

woodland was common, indicating that females were unlikely to attain range or core foraging area exclusivity.

7.4 - Discussion

Territoriality has been defined in various ways, a common one being the defence of a fixed spatial area to exclude other animals (Noble, 1939; Kaufmann, 1983; Maher and Lott, 1995). Territoriality can also be defined as the exclusive use of fixed space (Krebs, 1970; Kerr and Bull, 2006) or as a range which an animal has site-specific dominance (Emlen, 1957) and priority of access to resources over others, is achieved through social interactions such as advertisement behaviour and contests (Kaufmann, 1983). This 'exclusive use of fixed space' may refer to individuals, pairs or groups of animals defending an area and the degree of exclusive use varies enormously across taxa. How animals utilise space has important consequences for social organisation, foraging behaviour and mating. Territorial behaviour, in which a territory is defended by means of aggressive displays and/or threats, has been demonstrated empirically for many species of birds and fish (for review see Maher and Lott 2000). It has also been reported for some species of rodents (Beletsky and Orians, 1989) and primates (da Cunha and Byrne, 2006).

Studying territorial behaviour in bats is problematic because they are fast flying and nocturnally active so the likelihood of a researcher observing social interactions among conspecifics is low. One alternative for elusive mammals is to study the overlap in range use. Therefore, the amount of home range overlap or, conversely, range exclusivity is generally used as an index to infer territoriality in which territoriality is equated with 'little' overlap and absence of territoriality with 'more' overlap, with no set criterion of when a home range

becomes a territory (Maher and Lott, 1995). Bradbury (1977) concluded that female territoriality is virtually absent among bats, given the ephemeral nature of the distribution of prey items (Bradbury, 1977). However, Rydell (1986) described a scenario in which reproducing female northern bats *Eptesicus nilssoni* defended feeding territories against other colony members as well as non-members by means of aggressive chases and vocalisations (Rydell, 1986).

In this study, home range overlap was used as an index to infer the spacing patterns of females. The results of the current study show that the home range of *P.auritus* females may overlap considerably with one or more conspecific females. Overlap in female home ranges has been reported for various animals including coyotes *Canis latrans* (Chamberlain et al., 2000), black-footed ferrets *Mustela nigripes* (Jachowski et al., 2010), and eastern grey kangaroos, *Macropus giganteus* (Carter et al., 2009). However, other animals, such as European moles *Talpa europaea* (Macdonald et al., 1997), desert tortoises (Harless et al., 2009), and short-tailed mongoose *Herpestes brachyurus* (Jennings et al., 2010), do not exhibit such sharing tendencies amongst females, but often have range exclusivity.

Studies investigating spacing patterns in bats by means of radio-tracking have reported a large amount of variation in both range size and the extent of overlap between individuals. Table 7.5 summarises the findings of various radio-tracking studies on bats which have reported the home range overlap. The size of home range does not appear to be a good predictor for the extent of home range overlap, as species exhibiting large home ranges, such as the Barbastelle bat *Barbastella barbastellus* (Hillen et al., 2009), had low levels of overlap for radio-tracked females, as did Bechsteins bat *Myotis bechsteinii* (Kerth et al., 2001) whose

home ranges were small (Table 7.5). However, when making these comparisons, it must be acknowledged that, many of the studies, including this one, have only radio-tracked a small proportion of the bats. Therefore, the picture of the degree of overlap is likely to be incomplete.

Indirect evidence for overlap in the foraging areas of *P. auritus* has also been reported by Swift and Racey (1983), in which observations of bats marked with reflective tape recorded up to 'five *P. auritus* feeding together without any obvious interaction' (Swift and Racey, 1983). Entwistle (1994) simultaneously radio-tracked one pair of female *P. auritus* and found that they spent 11% of their time within the same feeding site. In addition, previous radio-tracking work indicated that there may be overlap in the feeding ranges of *P. auritus* (although the sample size was too small to be conclusive) (Murphy *et al.* unpublished data). Furthermore, as detailed in Chapter 4, 23% of filmed responses involved multiple individuals responding concurrently. This indicates that the individuals responding were likely to be in reasonable close proximity to one another when they were motivated to respond to the stimulus.

Some studies have reported animals overlapping in home range use but having exclusive core areas. For example, the dusky-footed wood rat *Neotoma fuscipes*, a nocturnal mammal, showed home range overlap with multiple neighbouring woodrats, both same sex and opposite sex, suggesting that foraging areas were shared. However, there was little overlap between same – sex neighbours in core foraging areas (Innes et al., 2009). Core area exclusivity has also been demonstrated for many animals including sleepy lizards *Tiliqua rugosa* (Kerr and Bull, 2006), wild ferrets *Mustela furo* (Norbury et al., 1998), and yellow-necked mice (Stradiotto et al., 2009). The simultaneously radio-tracked female *P. auritus*

clearly did not have exclusive use of their core areas, as the mean overlap in core area between dyads was 37.4%. Furthermore, the data on the non-simultaneously tracked females (radio-tracked over a four week period) at Small Dole suggests that *P. auritus* females may also exhibit extensive overlap in their core foraging areas in addition to their overlap in their foraging range. Overlap in core foraging areas (23-56%) has also been reported in the New Zealand long-tailed bat *Chalinolobus tuberculatus* (O'Donnell, 2001; Greaves et al., 2006). However, these studies used core foraging area estimates of 85% clusters as their range distributions were highly skewed.

Strong colony fidelity has been reported in various studies for *P.auritus* (Heise and Schmidt, 1988; Benzal, 1991; Entwistle, 1994) and, where such coloniality is coupled with female recruitment into the natal group and subsequent long-term philiopatry, colonies will be comprised of female kin originating from one or more matrilines e.g. (Wilkinson, 1985; Kerth et al., 2000; Rossiter et al., 2002). High relatedness amongst colony members may favour the evolution of cooperative behaviour, such as sharing resources within foraging areas, via kin selection (Hamilton, 1964). In this study, the relatedness levels of *P. auritus* were not assessed but a significant positive correlation between roosting association and foraging range overlap was found. All but one pair of dyads whose ranges overlapped roosted together at least once during the radio-tracking period, and the seven pairs that shared a roost during the brief radio-tracking period are, therefore, likely to have been from the same colony. Two of the three pairs that did not share a roost during the brief radio-tracking period also showed no overlap. It is possible that these two pairs were not from the same colony. A study on range overlap and roosting association in the tent-making bat *Artibeus watsoni* also found a significant positive correlation between range overlap and roosting association (Chaverri et al., 2007). Studies specifically examining relatedness as an explanation for the

extent of home range overlap have also found that the degree of overlap between individual foraging areas was significantly positively correlated with degree of genetic similarity (Kerth et al., 2001; Rossiter et al., 2002)

Radio-tracking studies that have involved simultaneously (or near simultaneously) recorded locations of two or more individuals have defined interactions occurring when two or more individuals are found within a critical distance of each other (determined by the radio-tracking regime). Such studies, for example on coyotes (Chamberlain et al., 2000), have found evidence of significant interactions whereby the authors interpret adult female coyotes 'positive interactions' as an indication that they were travelling together more often than expected by chance. A study on the interactions of grizzly bears *Ursus arctos* found that female-female interactions were more likely to occur at a greater frequency during a particular time of year (i.e. the berry season) (Stenhouse et al., 2005). By contrast, a study on the encounters of red foxes in Bristol found that, for most of the year, foxes practised avoidance and incursions into neighbouring territories were rare and, thus, conflicts were minimised, presumably because of the high risks involved with aggressive encounters (White and Harris, 1994). However, studies on the dynamic interaction of wild female ferrets *Mustela furo* (Norbury et al., 1998) and European moles *Talpa europaea* (Macdonald et al., 1997) have found no evidence of attraction or avoidance of simultaneously tracked dyads.

The dynamic interaction analysis, found no overall evidence of either attraction or avoidance for the simultaneously radio-tracked dyads. However, females radio-tracked in synchrony with juveniles did show evidence of cohesion. If these were mother-daughter pairs then this association would be expected, as studies of several species have found that females and their newly volant offspring frequently forage together (the yellow winged bat *Lavia frons*

(Vaughan and Vaughan, 1987), the big-brown bat *Eptesicus fuscus* (Brigham and Brigham, 1989) and the greater horseshoe bat *Rhinolophus ferrumequinum* (Rossiter et al., 2002)). Alternatively, it could be that juvenile *P. auritus* tend to associate more with adult individuals within their colony as juvenile northern long-eared bats *Myotis septentrionalis*, for example, have more direct and indirect associations with adult females, which may be a consequence of juveniles tending to be more exploratory (Patriquin et al., 2010).

The absence of an interaction between the female dyads that shared foraging sites may imply that the sharing of space, and resources within that space, is a common mechanism for female colony members. This study has demonstrated that although simultaneously radio-tracked pairs of females were spatially associated with one another, they were neither attracted to nor avoided one another when foraging. It is possible, however, that females occasionally came together attracted by calls to share particularly rich food sources. It could be that a female forages alone for the majority of the time but shows co-operative foraging tendencies by calling to advertise a rich food source, which in turn, attracts a conspecifc which heard her call and was motivated to respond. If this happened infrequently it may not be detected using radio-tracking methodology alone. However, an alternative explanation could be that females from the same colony are aware of one another's presence within their home range and can discriminate colony members from intruders, which may explain their propensity to respond to the Autobat stimulus.

Data on the distances between pairs of simultaneously radio-tracked females showed that 20% of the paired observations were within 50 metres of each other (a distance determined in Chapter 4 from where a *P. auritus* would reliably respond to the Autobat stimulus) and the majority of paired observations (approximately 78%) fall within the first five distance

categories (i.e. within 250 metres of the other female). The broadcasting of *P. auritus* simulated social calls within these foraging areas, and the resulting rapid response of one or more females, suggests that female *P. auritus* may co-operatively defend their shared resources against unknown intruders.

7.5 - Summary

- Female *Plecotus auritus* regularly shared foraging areas with conspecifics.
- For dyads simultaneously radio-tracked there was a significant correlation between roosting association and range overlap (i.e. dyads with greater overlap in their ranges roosted together more frequently).
- Although females regularly shared foraging areas, there was little evidence of co-ordination of movements between simultaneously radio-tracked dyads.

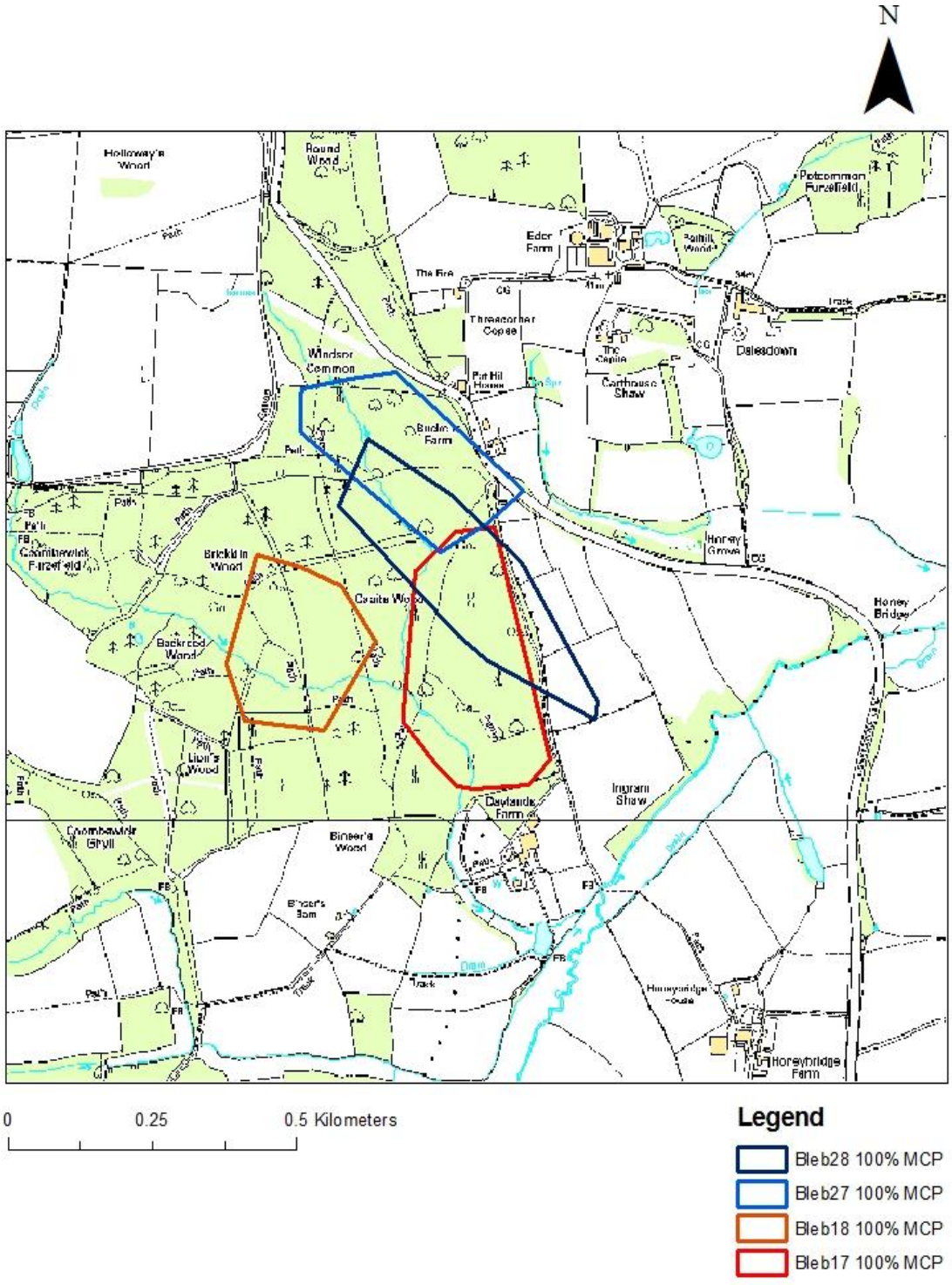


Figure 7.1: Range overlap of four female *Plecotus auritus* radio-tracked at Capite Wood, West Sussex in 2008. Bleb 17 (orange) and Bleb 18 (red) were caught at the same location and radio-tracked simultaneously. There was no overlap in the resulting 100% MCP for this pair of females. Bleb 27 (blue) and Bleb 28 (navy) were caught at the same location and radio-tracked simultaneously. There was overlap (29.13% of Bleb 27 100% MCP range and 24.32% of Bleb 28 100 % MCP range) for this pair of females and, also, overlap with Bleb 17 (not radio-tracked simultaneously).

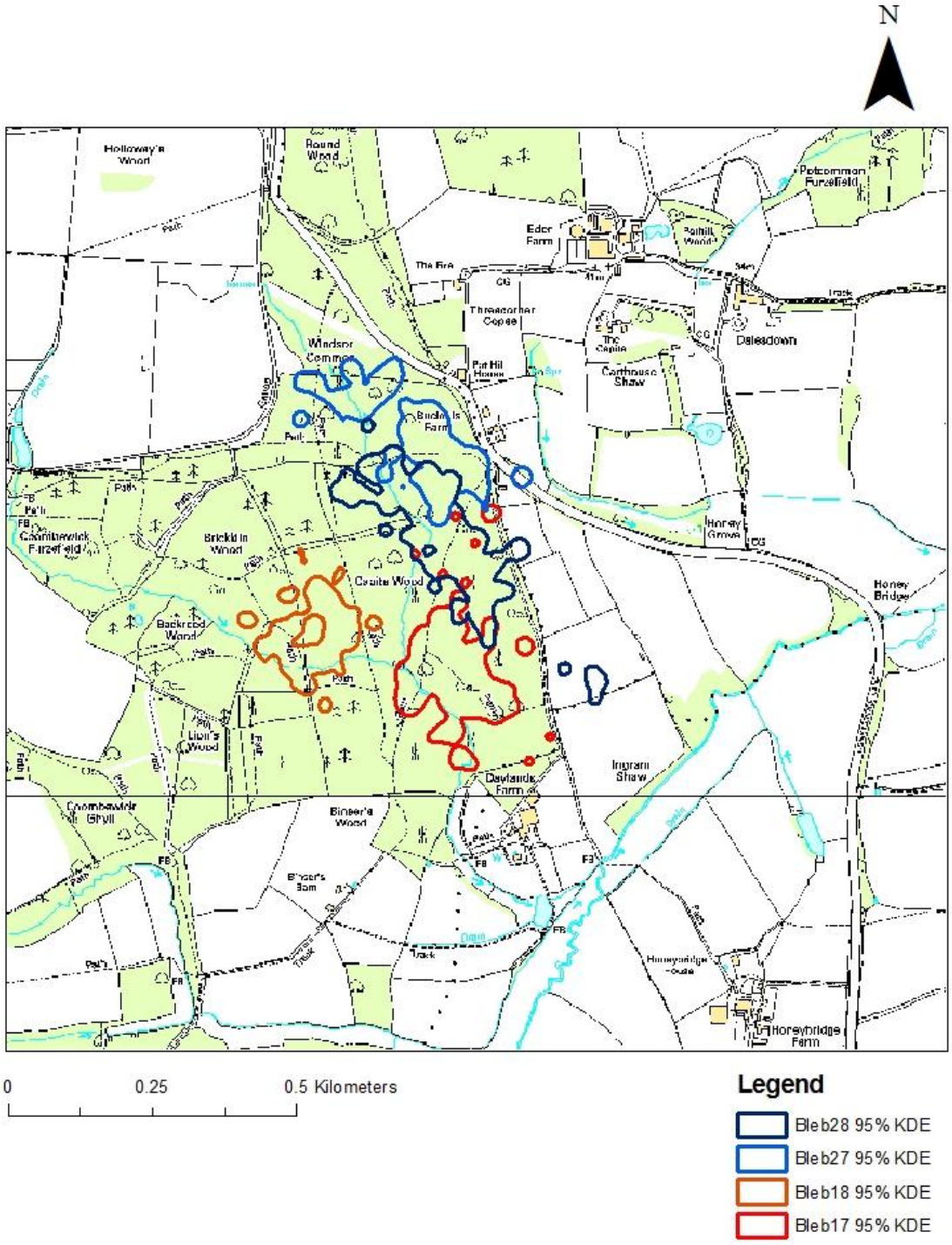


Figure 7.2: Range overlap of four female *Plecotus auritus* radio-tracked at Capite Wood, West Sussex in 2008. Bleb 17 (orange) and Bleb 18 (red) were caught at the same location and radio-tracked simultaneously. There was no overlap in the resulting 95% KDE for this pair of females. Bleb 27 (blue) and Bleb 28 (navy) were caught at the same location and radio-tracked simultaneously. There was overlap (24.05% of Bleb 27 95% KDE and 24.32% of Bleb 28 25.05 95 % KDE) for this pair of females. Bleb 28 also overlapped with Bleb 17 in the 95% KDE (not radio-tracked simultaneously).

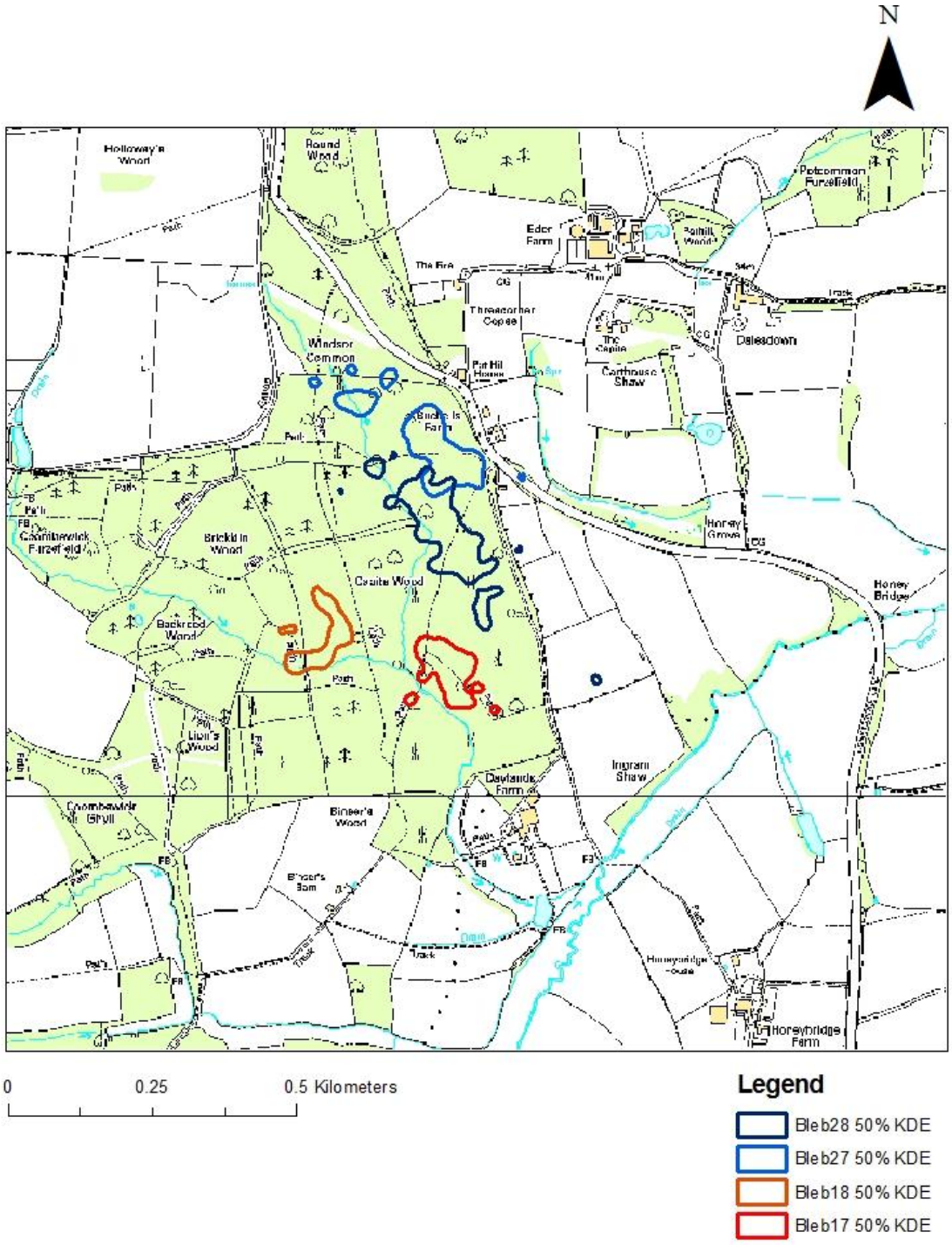


Figure 7.3: Range overlap of four female *Plecotus auritus* radio-tracked at Capite Wood, West Sussex in 2008. Bleb 17 (orange) and Bleb 18 (red) were caught at the same location and radio-tracked simultaneously. There was no overlap in the resulting 50% KDE for this pair of females. Bleb 27 (blue) and Bleb 28 (navy) were caught at the same location and radio-tracked simultaneously. There was overlap (17.54% of Bleb 27 95% KDE and 19.23% of Bleb 28 95 % KDE) for this pair of females.

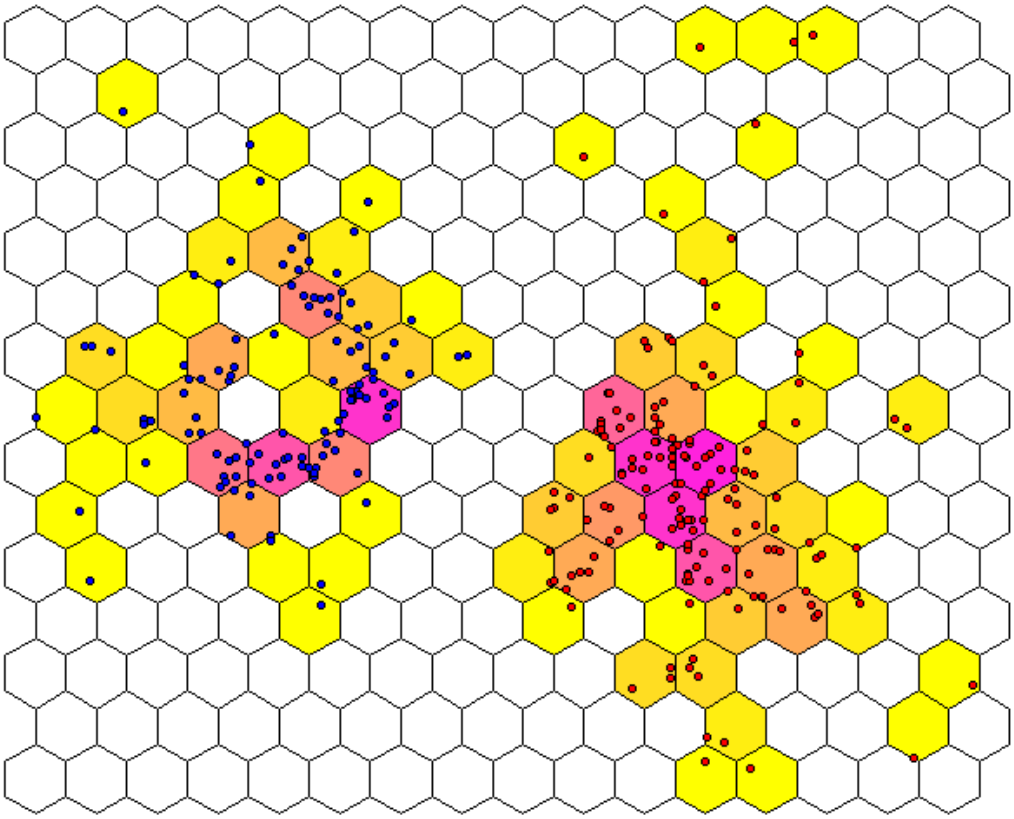


Figure 7.4 (a): Spatial association of the estimates of location (fixes) of two females (pair 3 – bleb 17 & 18), radio-tracked simultaneously at Capite Wood. The estimates of location for bleb 17 are shown by the red dots and the estimates of location for bleb 18 are shown by the blue dots. The Schluter test calculates a grid system over the estimates of location with the lighter colours (yellow) representing areas with 1 or 2 fixes (areas used less frequently) and the darker colours (pink) representing the clumping of fixes (or core areas). The number of grids where locations occur for both females, in this dyad is 0 and, therefore, the Schluter test has calculated a negative spatial association (X_2 7.821, n = 273, p > 0.005) and the corresponding Jaccard index is 0 (p < 0.001), as no grid squares had locations for both animals.

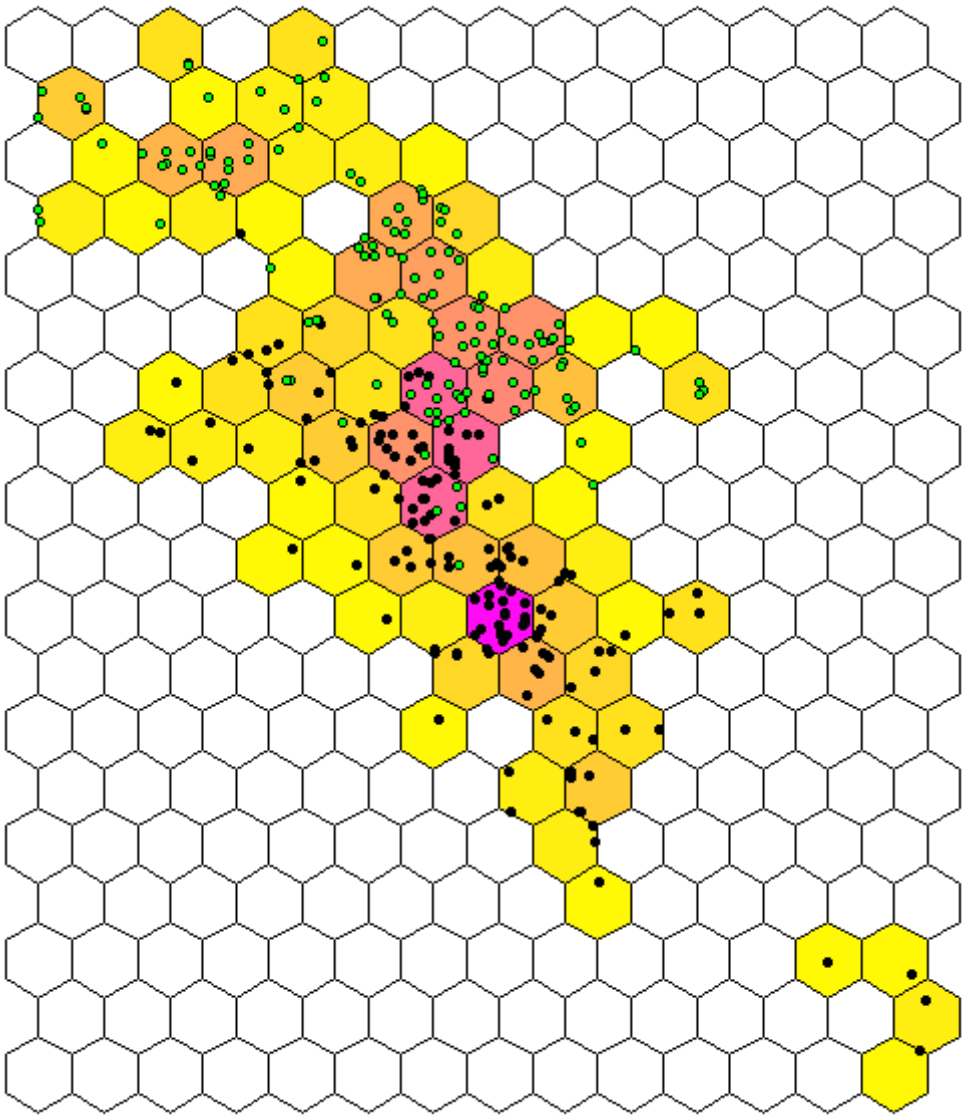


Figure 7.4 (b): Spatial association of the estimates of location (fixes) of two females (pair 8 – bleb 27 & 28), radio-tracked simultaneously at Capite Wood. The estimates of location for bleb 27 are shown by the green dots and the estimates of location for bleb 28 are shown by the black dots. The Schluter test calculates a grid system over the estimates of location with the lighter colours (yellows) representing areas with one or two fixes (areas used less frequently) and the darker colours (pinks) representing the clumping of fixes (or core areas). The number of grids where locations occur for both females, in this dyad is 9 and, therefore, the Schluter test has calculated a positive spatial association (X_2 11.564, n = 302, p > 0.021) and the corresponding Jaccard index is 0.118 (p < 0.01), as 9 grid squares had locations for both animals. The grids of spatial association where both occur more frequently (with clumping i.e. darker colours) are given greater weighting than the grids where there may be only one or two fixes for each animal.

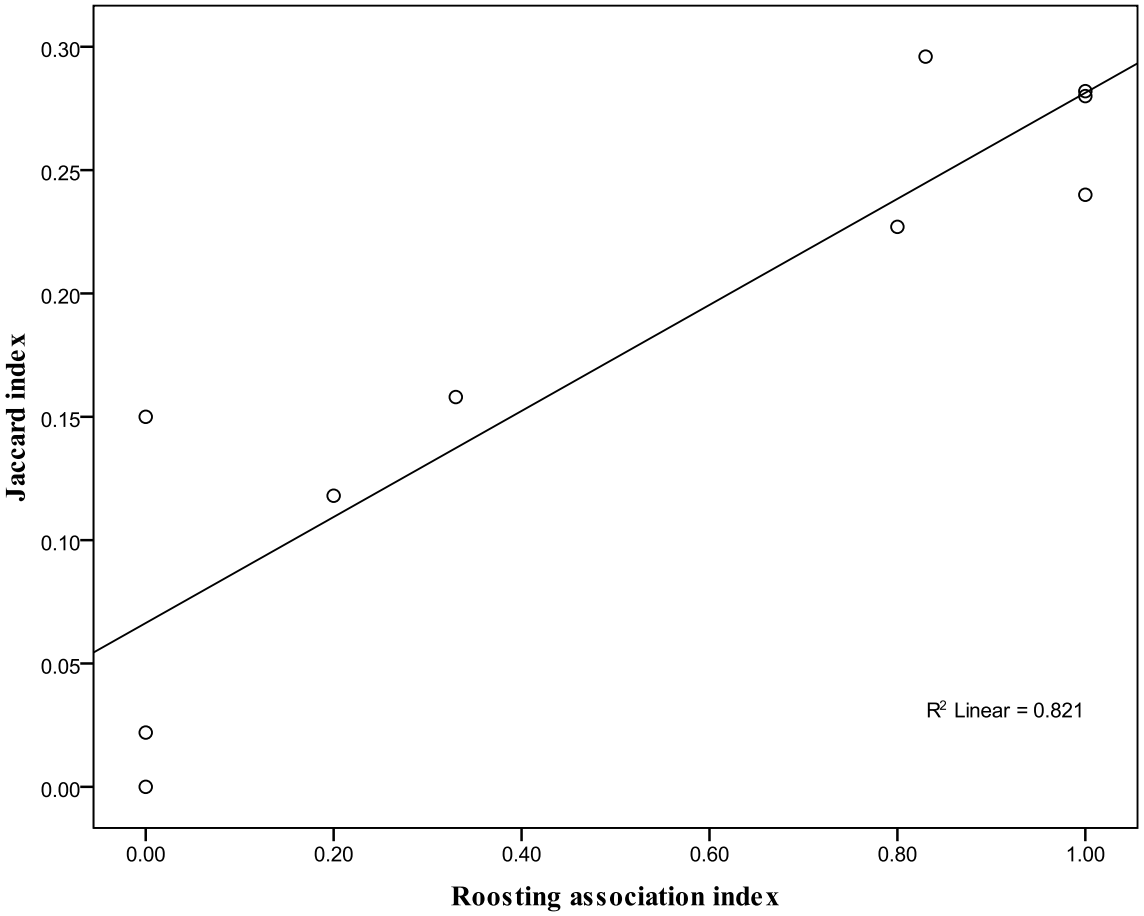


Figure 7.5: Correlation between the roosting association index and overlap of home ranges as measured by the Jaccard index.

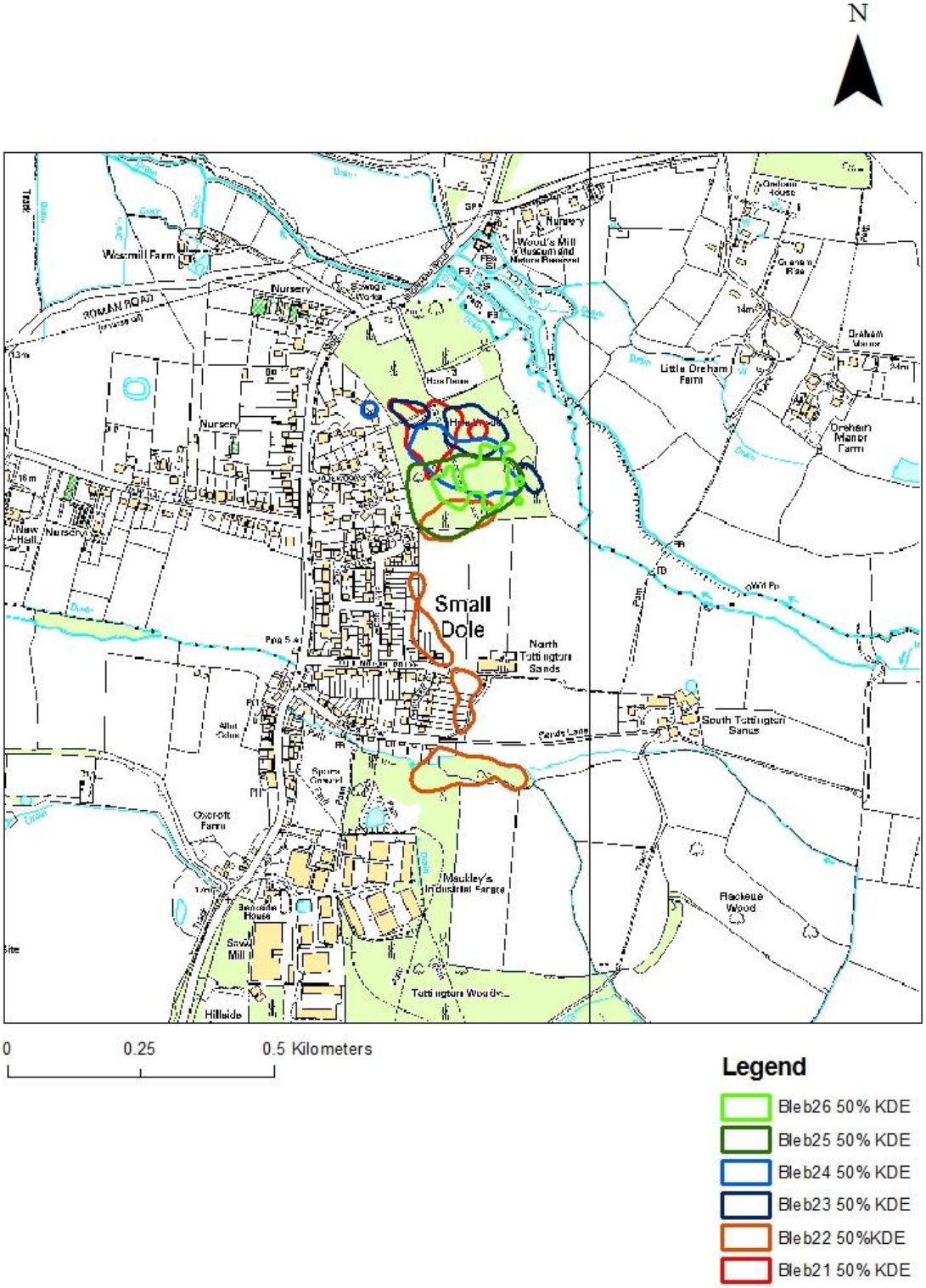


Figure 7.6: Core foraging areas (50% KDE) of six female *P.auritus* radio-tracked from 14th July 2008 to the 11th August 2008 at Hoe Wood.

Species	Habitat	Number of bats radio-tracked	Home range estimator	Mean Range size (ha)	Overlap (%)	Radio-tracked simultanously	Radio-tracked majority of colony	Source
Tent- making bat *Artibeus watsoni*	Primary and secondary tropical forest	42: 26♀ 16♂	100% MCP	3.6	47%	Yes	All individuals in known roosts	Chaverri, G., et al. (2007a); Chaverri, G., et al. (2007b)
Greater sac-winged bat *Saccopteryx bilineata*	Primary and secondary tropical forest	16: 8♀ 8 ♂	100% MCP 95% Kernel 50% Kernel	22±17; 12±6; 1.7±0.9	57 ± 22 (females overlapped with males)	Yes - ♂/♀ dyads	No - only male - female pairs from eight known roosts	Hoffman et al (2007)
Common pipistrelle *Pipistrellus pipistrellus*	Agricultural with riparian woodland	14♀	100% MCP Foraging MCP 75% Kernel	c.200; c.175 c.25	c.36 c.27 c.11	No	No	Nicholls & Racey (2006)
Soprano pipistrelle *Pipistrellus pygmaeus*	Agricultural with riparian woodland	12♀	100% MCP Foraging MCP 75% Kernel	c.60; c.35 c.15	c.24 c.18 c.12	No	No	Nicholls & Racey (2006)
Egyptian slit-faced Bat *Nycteris thebaica*	Acacia savanna with patches of riparian forest	18: 8♀ 8 ♂	95% MCP	13.7	49%	No	No	Monadjem et al (2009)
Barbastelle *Barbastella barbastellus*	Deciduous oak forest interspersed with meadows and brooks	12♀	95% Kernel 50% Kernel	403 67	13 3.2	4 dyads	No	Hillen et al (2009)
Bechstein's bat *Myotis bechsteinii*	Mixed deciduous Beech forest	12♀	100% MCP	21	12.3	Yes - dyads	c.50%	Kerth et al (2001)
Long-tailed bats *Chalinolobus tuberculatus*	Temperate rainforest	50: 28♀ 10 ♂ 12 juvenile	100% MCP	1589 ♂, 1361 post-lac ♀, 657 non-repro ♀	31-68	No	c.50%	O'Donnell 2001
Brown Long-eared bats *Plecotus auritus*	Broadleaved deciduous woodland	24♀	100% MCP 95% Kernel 50% Kernel	51±5; 53±6; 47±6		8 dyads	No	This study

Table 7.5: Studies reporting home range overlap in bats by means of radio-telemetry. Only one study on the Tent-making bat *Artibeus watsonii* radio-tracked all individuals within a known roost. None of the studies radio-tracked all bats present so the range overlap values presented are likely to be an incomplete picture.

8.1 – Conclusions from this study

The aim of this thesis was to examine the structure and function of social calls in Brown Long-eared bats *Plecotus auritus*. Some studies have described the structure of specific *P. auritus* call types from sonograms (Ahlen, 1981; Furmankiewicz, 2004). However, only one study to date has examined the function of *P. auritus* social calls, and this investigated at isolation calls given by infants separated from their mothers (Defanis and Jones, 1995). However, bats produce a variety of different calls in different contexts (Pfalzer and Kusch, 2003) that are likely to have a variety of functions. This research set out to investigate some aspects of social call structure and function using a combination of recording and playback techniques.

The first step was to characterise a range of calls given by *P. auritus* in the vicinity of maternity roosts. Social calls are given very frequently in this context. Calls were systematically recorded across 20 summer maternity colonies in Sussex and the acoustic variation was examined. Initial visual examination of sonograms revealed that there were three distinct groups of social calls which could be reliably distinguished on the basis of their gross structure. Almost 97% of the calls had a similar basic structure, an FM sweep descending in frequency from (mean values) 52kHz to 14kHz, ending with a short (1 ms) QCF, typical of the *P. auritus* social calls described by Ahlen (1981), Russ (1999) and Furmankiewicz (2004). Calls with this similar basic structure were subsequently termed 'Type A' calls. When the acoustic parameters of Type A calls were extracted, model-based cluster analysis revealed that this group could be subdivided into six clusters. These were subsequently named calls one to six. Markov chain analysis revealed that there was no evidence that these call were emitted in a specific order, only that similar call types (calls

from the same cluster) tended to be repeated. However, the Markov chain analysis conducted in this study was limited to the analysis of couplets and it may be that higher order sequences do exist.

The remaining (approximately 3%) calls had a different gross structure and these calls were termed Call 7 and Call 8. Call 7 was characterised by an ascending FM sweep followed by a descending sweep. Call 8 was characterised by a descending FM sweep followed by an ascending FM sweep. Both of these call types showed a showed a linear increase in occurrence throughout the seasons and were also recorded significantly more frequently in September. This increase in occurrence was greater than the increase for any of the Type A calls in September. Call 7 had the same structure as the Autobat output 'stimulus C' (Chapter 4). Responses of *P. auritus* to this stimulus also occurred significantly more frequently in September. These seasonal patterns suggest that the function of the call may be seasonally specific. One explanation would be that these calls are related to mating. Burland et al., (2001) found that male *P. auritus* resident in maternity colonies are philopatric. However, most offspring are fathered by males from outside the colony and it may be that sexually active non-resident males begin to visit maternity roosts at the onset of the mating season. The patterns of vocal production at maternity roost sites (Chapter 3) showed an increase in the number of social calls at roost sites in September, despite the decrease in the number of bats present in the colony. This suggests that there are fewer bats vocalising more frequently, and the roosts could, perhaps, be utilised as mating roosts when the maternity colonies begin to disband. Furmankiewicz (2002; 2008) also described this call type at *P. auritus* underground swarming sites in Poland in early spring when *P. auritus* mate after winter hibernation.

It was often clear from sonograms that more than one bat was producing social calls. It may be that one function of calling around the roost is to strengthen social bonds between members of the colony. This pattern of behaviour may account for the observed correlation between colony size and the number of social calls recorded. The fact that social calls were recorded significantly more frequently in the quarter of the night before sunrise also supports this hypothesis.

There was also no evidence to suggest that certain call types were specific to individual roosts or geographic locations. All of the six Type A calls were recorded at all of the 20 maternity roost sites. Calls 7 and 8 were recorded at most of the maternity roosts across all of the three locations. Therefore, it was concluded that these types of calls could not be roost or location specific. However, this study did not analyse within-call differences and there may be differences in acoustic parameters at a finer scale between roosts and populations.

In the majority of the thesis, I have presented the results of responses to simulated social calls produced by the Autobat to answer questions about the function of *P. auritus* social calls. (Chapters 4, 5, 6 and 7). One of the central questions of the thesis was 'why do bats respond to the Autobat'? Playback is the technique of re-broadcasting natural or synthetic signals to animals and observing their response (McGregor 1992). Studies researching the vocal communication in animals have used playback as a tool to investigate both vocal recognition of conspecifics (Harrington, 1986; Insley, 2000) and call function (Harrington, 1987; Barlow and Jones, 1997). However, playback studies in the field also have limitations in that the responses given are dependent not only on the signal itself but also on the context including both social and ecological factors (McGregor 1992). Playback studies often broadcast the calls more frequently than they would occur under natural conditions in order to elicit a

response from the focal animals, and this use of playback has been referred to as 'super-stimulus'. For example, a study on three bat species that investigated the influence of echolocation call design and foraging strategy on the role of echolocation calls in communication used a playback of 51 repeated feeding buzzes as a 'super-stimulus' (Balcombe and Fenton, 1988).

The Autobat output can also be regarded as a 'super-stimulus' as it repeats sequences of sweeps at much higher rates than they have been recorded in the field. However, if the Autobat output was modelled to occur at a rate that resembled the normal frequency of occurrence of social calls, then it would be extremely difficult to obtain sufficient results with respect to the frequency of response due to very low frequency of calling and the highly mobile nature of bats. However, it raises the possibility that the bats' response may have nothing to do with the similarity of the stimulus to a conspecific's social calls, but that they may be responding to ultrasonic disturbance in the environment. This was tested by the capture experiment (Chapter 4), that compared the bat's responses to simulated calls of their own species, another species and mechanical noise of a comparable duration and bandwidth. *P. auritus* were captured significantly more frequently to simulations of their own social calls. This suggests that the response of *P. auritus* to the stimulus is a social one, elicited by the similarity of the stimulus to conspecific social calls.

Once I had established that the similarity of the stimulus to a *P. auritus* social call influenced the propensity of bats to respond, I then (Chapter 4): (i) assessed whether the responses varied seasonally and between sexes; and (ii) analysed the nature of behavioural responses elicited by a range of stimuli modelled on various social calls of their own species. There was

evidence of a seasonal difference in the responses to different stimuli whereby high repetition high intensity calls elicited more responses earlier in the season (June) compared to later in season (August / September). Females were captured significantly more frequently earlier in the season compared to later when males were caught more than females. Furthermore, the behavioural responses observed on infra-red video in June were more complex, longer in duration, and occurred more frequently than later in the year.

One of the hypotheses put forward to explain the bat's response to the stimulus is that the responses are related to defence of the foraging area. In order to test this hypothesis, the activity patterns and the extent of home range use was ascertained for 24 female *P. auritus* across a variety of woodland sites in Sussex (Chapter 5). The study subsequently examined the responses of females in relation to home range use by varying the stimulus location within the bat's foraging range (Chapter 6). A novel technique was used to identify responding individuals and the study found that females were more likely to respond to simulated social calls played in their core foraging range than in their peripheral foraging range or outside of their range. The propensity of *P. auritus* to respond maximally to simulated social calls in core foraging areas is consistent with the resource defence hypothesis.

One alternative explanation to the resource defence hypothesis is that the response to the stimulus represents coordination of foraging by attracting other colonies members to resources. Social calls can serve to attract and recruit conspecifics to food (Wilkinson and Boughman, 1998; Pollick et al., 2005; Dechmann et al., 2010). This hypothesis was explored by examining overlap in range use amongst females radio-tracked in the same woodland and, by also assessing the interactions of pairs of bats by simultaneously radio-tracking 10 female

dyads. There was extensive overlap in range use for adjacent pairs of females radio-tracked at the same woodland site. Given that only a very small proportion of each of the colonies was radio-tracked at each site, the results suggest that females are likely to share with multiple other females. The interaction analysis indicated that most adult female pairings were neither attracted to nor avoided one another when foraging, but adult–juvenile pairings were positively associated more frequently than would be expected by chance. Furthermore, dyads that had greater overlap in their ranges, also roosted together more frequently.

Adult females from the same colony may be aware of one another's presence when foraging and may be able to discriminate colony members from intruders. There was, however, no evidence of roost specific call signatures (Chapter 3). Even though the location within the foraging range from which the stimulus is broadcast clearly influenced the propensity of females to respond, based on the results of this study it cannot be completely ruled out that the response to stimulus represents co-ordination of foraging. *P. auritus* females may forage alone for most of the time but show co-operative foraging tendencies by calling to advertise a rich food source, which attracts one or more conspecifics. If that were the case, however, it may be expected that there would be no difference in response in relation to whether the call came from the core or the peripheral foraging area.

A further question which is important to address is whether the Autobat is a legitimate way of conducting research on the function of bat social calls. The use of synthesised calls produced by the Autobat has some distinct advantages over the playback of recordings of social calls. It is extremely difficult to get high quality recordings of brown long-eared bat social calls away from roosts, as they tend to produce social calls infrequently and unpredictably. This means that calls must be recorded opportunistically and recording conditions tend to be poor, with

weak signals and excessive background noise. While such recordings are not suitable for playback, as long as the time and frequency coordinates of the call can be accurately measured from a sonogram, the calls can be used to produce very clear output from the Autobat. Also, the use of artificial stimuli allows for the control of differences between stimuli and avoids potential problems of pseudo-replication inherent with playback studies (for review see Kroodsma et al., 2001). Furthermore, the equipment required for playback is also more expensive and fragile than the Autobat, making it more difficult to use under field conditions. This is especially important for experiments that require frequent relocation of the equipment in woodland at night.

On the other hand there are potential drawbacks to using synthesised call stimuli. The main one is that the synthesised calls are artificial sounds, and it may be that differences in acoustic quality between the stimuli and actual social calls influence the way that bats respond to them. However, similar problems may well occur with playback of recorded vocalisations. Even with flat response microphone and speaker, it is likely that there will be acoustic differences between the original call and the reproduced call. One is that the recorded call will have undergone some attenuation between bat and microphone. The playback output will then undergo further attenuation between the speaker and the bats that hear it, and so the intensity of playback will be reduced, and particularly that of the high frequency portions of the call. Also, naturally emitted calls are given by bats that are in flight, and so the sound source is moving, not only in terms of whole body movements, but also movements of the head in relation to the body. This is likely to alter sound quality in ways that will not be simulated by a fixed speaker.

8.2 – Suggestions for future research raised by this study

This has been the first study to attempt to quantitatively classify a variety of social calls of *P. auritus* and investigate responses to them. However, one of the problems with conducting a field study in this manner is that there is very little data on the identity of the caller and, also, it was often not known who was responding to the stimulus calls (with the exception of the individuals that were marked; Chapters 4 and 5). In a laboratory setting this aspect could be controlled for by recording callers and watching the responses of known individuals. The following discussion examines how each of the key areas I researched could be expanded upon and improved.

(i) It was possible to objectively classify individual social calls by recording calls at the roost sites and subjecting these calls to statistical classification techniques. However, based on recordings from the field, it is likely that individuals often produce social calls in a sequence, as opposed to singularly. Nonetheless, the first order Markov chain analysis did not find any evidence that one particular call type followed another type. In Chapter 3, however, it was demonstrated that the number of social calls at roost sites was significantly correlated with the number of bats roosting in the maternity colony and one explanation for this trend could be that the majority of bats are contributing to the vocalisations. Consequently, interpretation of whether calls are produced as sequences of vocalisations is difficult because multiple vocalisations from different individuals would confound the results. More controlled studies than are possible in the field using a laboratory based approach may be required in order to fully investigate and quantify whether *P. auritus* produce vocalisation as sequences, as demonstrated for other species of bat by researchers under laboratory conditions (Kanwal et al., 1994; Bohn et al., 2009).

(ii) Experiments with a temporary captive colony of bats could also address the question of whether the Autobat is a legitimate manner in which to conduct research on bat social calls. The best way to assess whether the artificial nature of synthesised or playback calls is influencing the nature of the bats' responses would be to observe their responses to naturally emitted calls. This would be impossible to do in a field situation with free-flying bats, but it may be possible to gain some insight from observation with bats in captivity, if they produced social calls sufficiently frequently. This would also allow for experimental comparison of bats' responses to output from the Autobat and playback of recorded social calls.

(iii) With bats in temporary captivity it would also be possible to examine whether individual identity is encoded within social call structure. Individual recognition is important for roost members to recognise roost associates. If males are present in roost then it is important to recognise resident males from males of adjacent populations. If, for example, the Autobat were used to elicit vocal responses from known captive individuals, and these responses were recorded, these could then be played back to subjects to examine how responses differ to different kinds of calls. It would also be possible to examine how responses differ to calls of roost mate and non-roost mates. This could also be combined with DNA analyses to examine whether responses to kin differ from those to non-kin colony members. By using infra-red video and ultrasound recording it would be possible to see how colony members of both sexes approach the stimulus.

(v) An alternative hypothesis that could be examined as to why bats vocalise at roosts sites, and in particular vocalisations in the period before dawn, could be that social calling is related to the group decision making process with respect to roost choice. Studies have demonstrated that bats roosting in trees frequently switch roost sites (Lewis, 1996; Russo et

al., 2004; Willis and Brigham, 2004; Vonhof and Betts, 2010), and radio-tracking *P. auritus* roosting in trees in Sussex also demonstrated that this species frequently switched roost during the radio-tracking period (Murphy et al in prep). Although the social calls recorded in this study were from bats roosting in buildings, in the absence of human influence *P. auritus* would have roosted in trees and vocalising at roost sites may be inherent in the group decision-making process of deciding on the tree in which to roost. Using vocalisations in the context of movement decisions has been demonstrated for group living species such as meerkats *Suricata suricatta* (Bousquet et al., 2011), primates (e.g. white-faced capuchin, *Cebus capucinus* (Boinski and Campbell, 1995)), and birds (e.g. green woodhoopoes *Phoeniculus purpureus* (Radford, 2004)). It would be instructive to examine if there were differences between calls at synanthropic roosts and tree roosts. An intensive study of tree-roosting colonies, which switch roosts very frequently, may help to elucidate the function of calls in relation to roost switching. This could be achieved using a combination of radio-tracking, recording behaviours with infra-red video and recording their vocalisations.

(vi) Call 7 were frequently recorded at maternity roost sites later in the season (September) compared to earlier in the season (May – June). They had the same call structure as stimulus C (ascending and descending FM sweep) to which bats responded significantly more frequently at foraging sites. One hypothesis proposed for this call type is that the call may be related to mating and an experiment that could specifically address this hypothesis would be to playback simulations of this call type and to capture the individuals responding. Comparing the sex ratio and whether the males responding were sexually mature may indicate whether the calls play a role to function in mate attraction. It would also be interesting to ascertain whether there is sexually mature non-philopatric males present at maternity roost sites in September and how, if at all, these males are related to females

present in the colony in September. If summer maternity colonies also function as mating colonies for *P. auritus* this may have important implications for their conservation.

(vii) One suggested explanation for bats response to the stimulus calls was that the responses represent defence of resources. This hypothesis was supported by the result that females responded maximally to the stimulus in their core foraging area. If the resource defence hypothesis is true then it would be interesting to examine whether females maintained this response over time and if their ranges and / or responses to the stimulus changed seasonally or even between years. Resources availability may change from season to season or from year to year, prompting animals to shift their home range, make seasonal excursions, or alter patterns of social organisation (Lott, 1991). Radio-tracking a number of individuals repeatedly, within and between years, to ascertain how or if their home range changes will provide insight into how fixed or flexible the system is.

(viii) Although there was little conclusive evidence from the radio-tracking data that adult females dyads were interacting when foraging, one way to further investigate whether there is any evidence of co-ordinated responses to the stimulus would be to try to catch three or four females in the same area, ascertain their home range, and to then present them with stimulus calls in relation to their home range use and investigate whether there is any co-ordination in responses using a combination of radio-tracking and infra-red video. This approach would require a number of field-workers to simultaneously radio-track and locate females, but it is certainly a feasible approach given adequate resources. If only one female responds, the female who primarily occupies that part of the range, then this could indicate that the female is defending resources within her range. If a female shares her range with one or more females, do all females respond?

Finally, on a practical note, understanding why bats respond to the stimulus is important for developing the system as a survey tool. In the UK, for example, the Autobat is currently used by the Bat Conservation Trust to systematically survey for Bechstein's bat *Myotis bechsteinii* and has also been used for systematic species surveys and experiments in other parts of Europe (Goiti et al., 2008; Boston et al., 2010; Schöner et al., 2010). The data and experiments detailed in Chapters 4 and 6 demonstrated that *P. auritus* rapidly approach the source of the stimulus broadcast. However, repeatedly broadcasting simulations of social calls may inadvertently affect an individual's fitness by deterring it from its preferred foraging locality. Therefore, it is important that the use of the Autobat as a survey tool is conducted in a controlled manner whereby targeted areas are not 'over-surveyed'.

Although the work carried out in this thesis has shed some light on the function of social calls in *P. auritus*, many further issues remain to be investigated. More controlled studies than are possible in the field may be required if the functions of social calling are to be thoroughly investigated and quantified.

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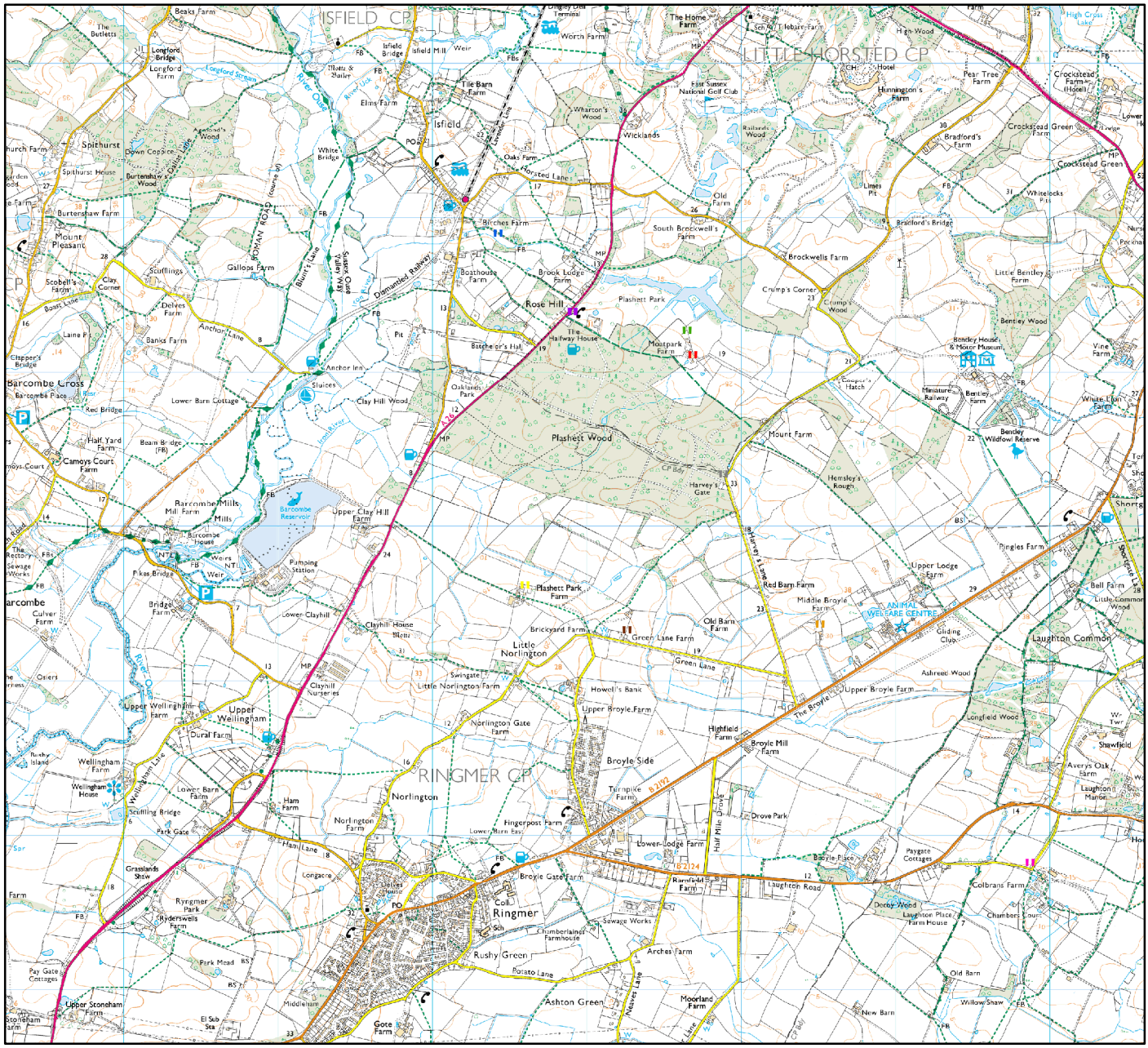
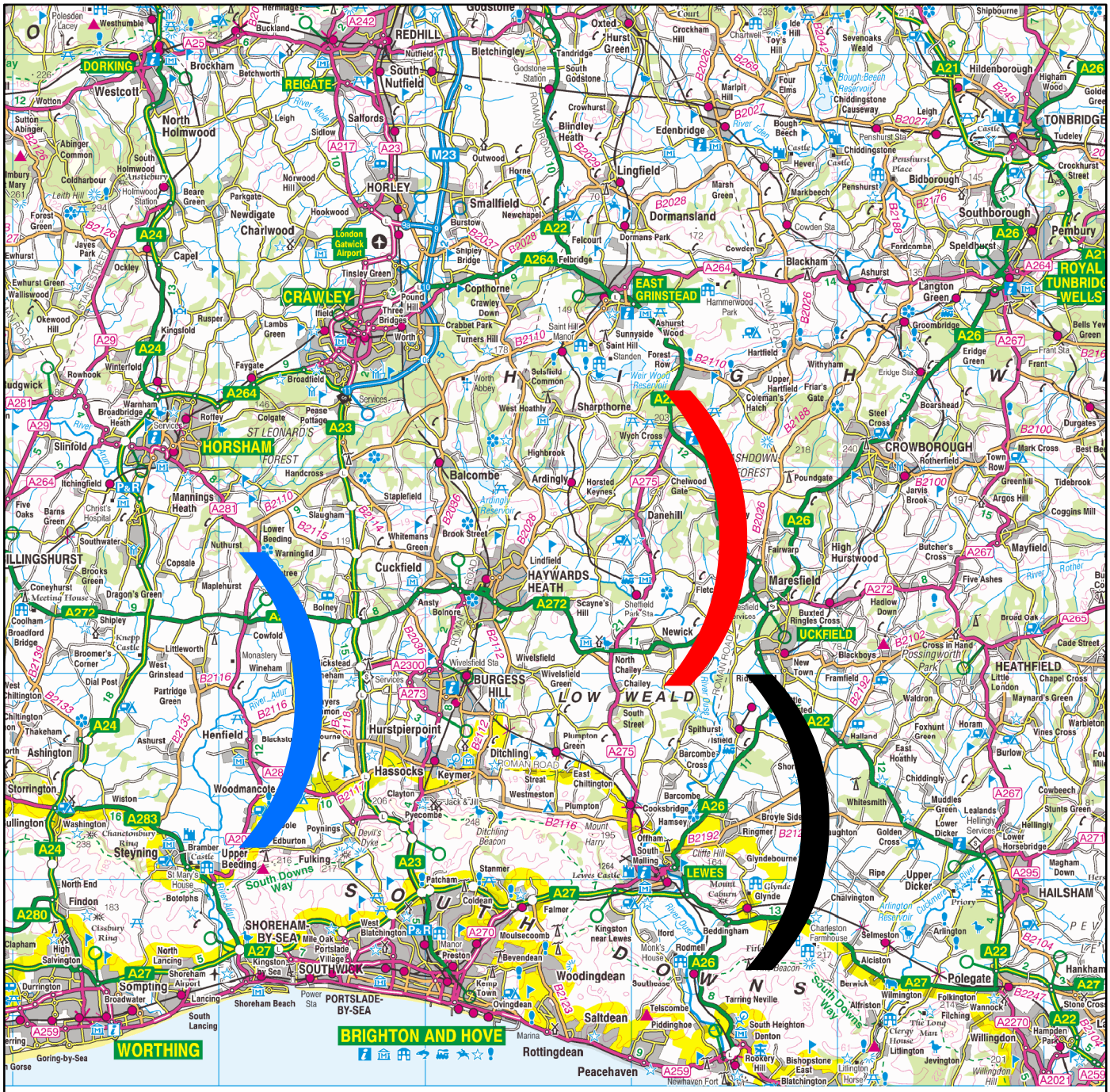


Legend

- B1: Middle Broyle Farm
- B5: Plashett Park Farm
- B6: Harveys Gate
- B8: Moat Park Farm
- B10: Colbrans Cottages
- B20: 2 Plashett Park Gate
- B21: 3 Plashett Park Gate
- B23: Moat Park Cottage
- B26: Green Lane Farm
- B28: Oast House Isfield

Appendix A.3.1: Roosts around the Plashett Wood Area North of Lewes.
Two roosts, 2 & 3 Plashett Park Gate, are situated in the same row of cottages (but are not physically connected). These roosts are denoted by the one purple marker.



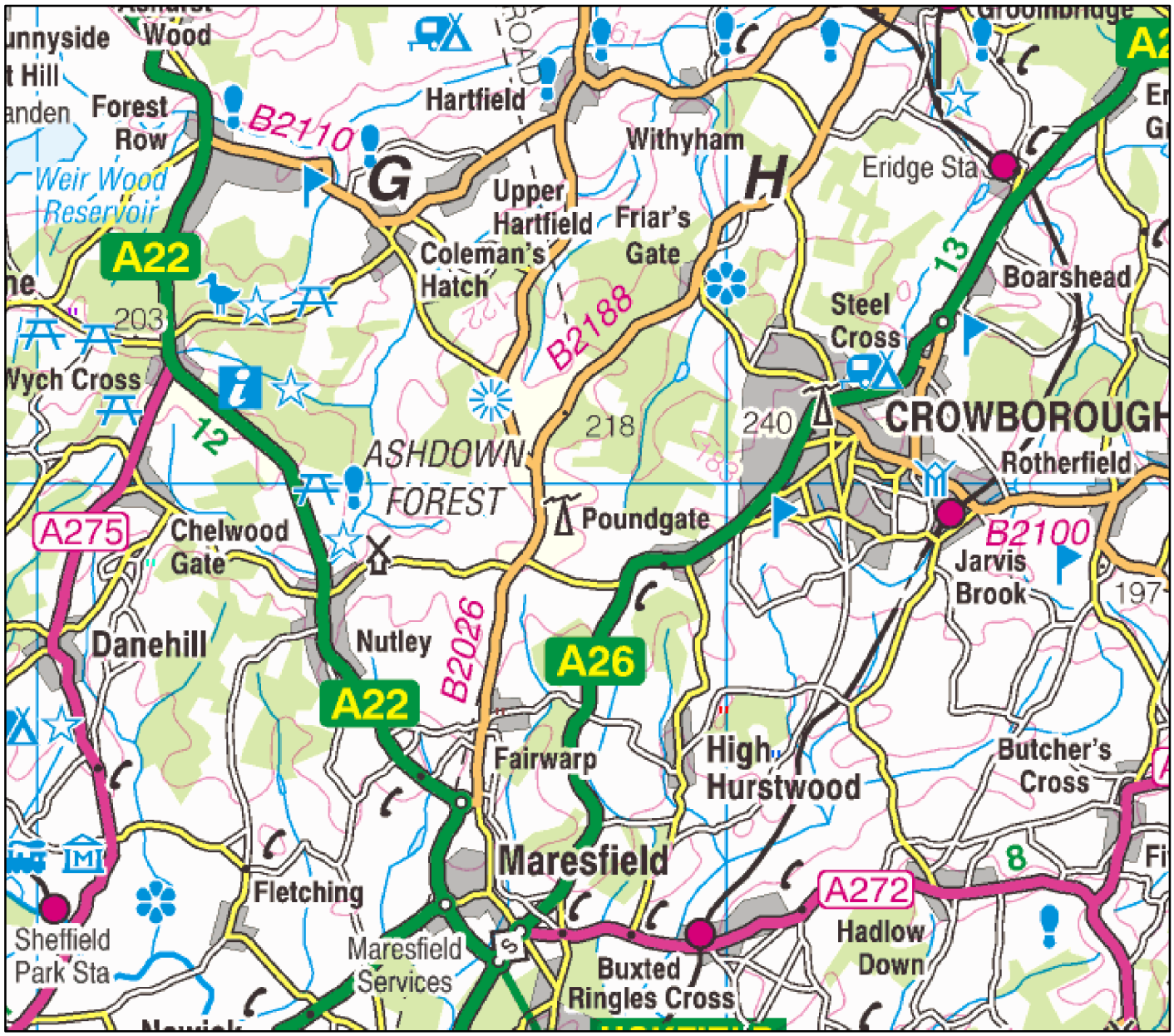
Legend

) **Area 1: East Sussex North-east of Lewes**

) **Area 2: East Sussex Ashdown Forest**

) **Area 3: West Sussex Cowfold Area**

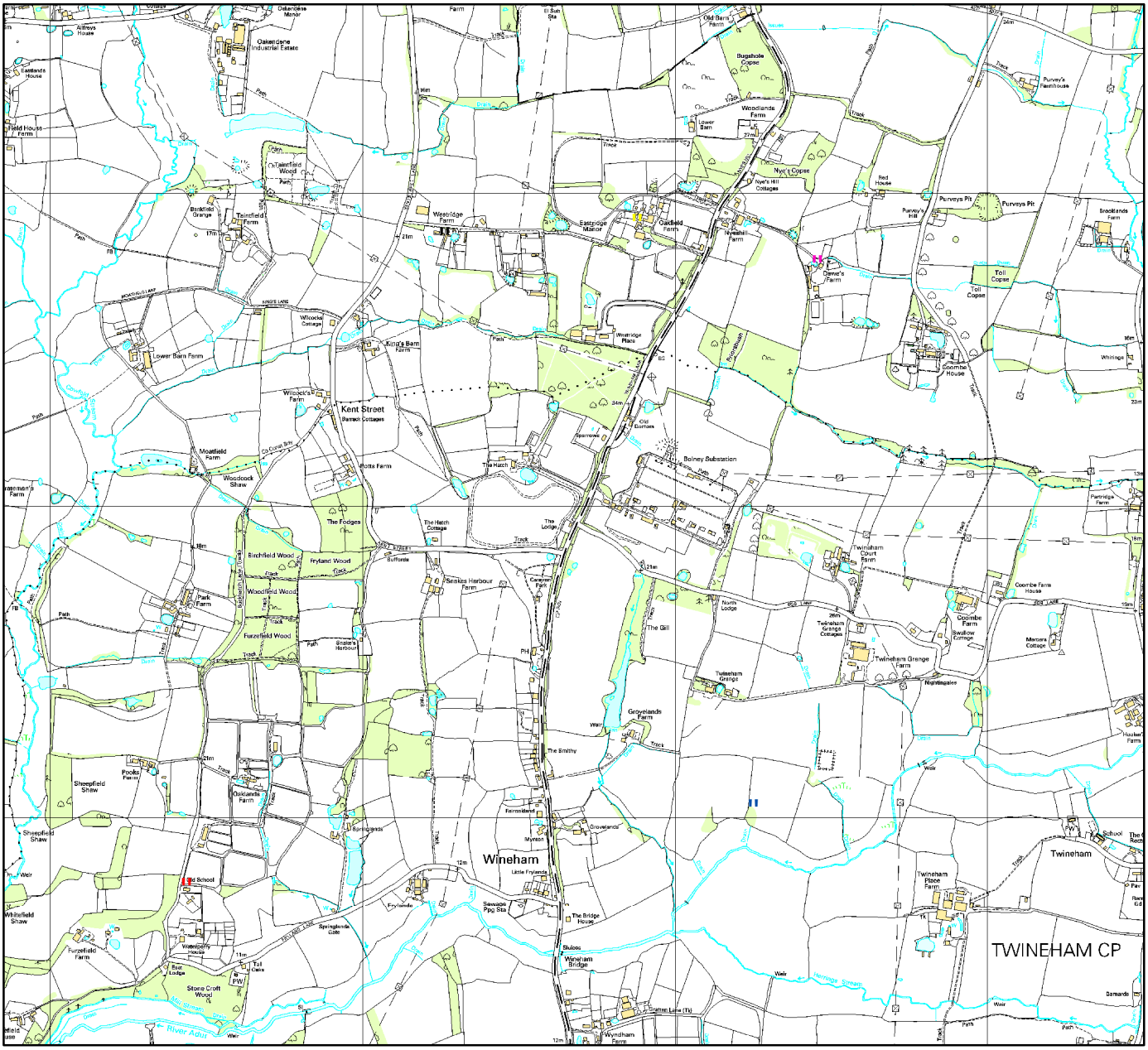
Appendix A.3.2: The three areas in Sussex identified for the study of Brown Long-eared social calls at roost sites.



Legend

- B2: Sleeches Farmhouse
- B4: Pickreed Cottage
- B9: Lantern Cottage
- B13: Charlwood Gate
- B16: Springfield Cottage

Appendix A.3.3: Locations of Brown Long-eared roosts in the Ashdown Forest Area, East Sussex, used in the study.



0 0.25 0.5 1 Kilometers

Legend

- B3: Oakfield
- B7: Kings Kent
- B15: Old School
- B22: Oakwood Farm House
- B27: Dawes Farm

Appendix A.3.4: Locations of Brown Long-eared roosts in the Cowfold area, West Sussex used in the study.

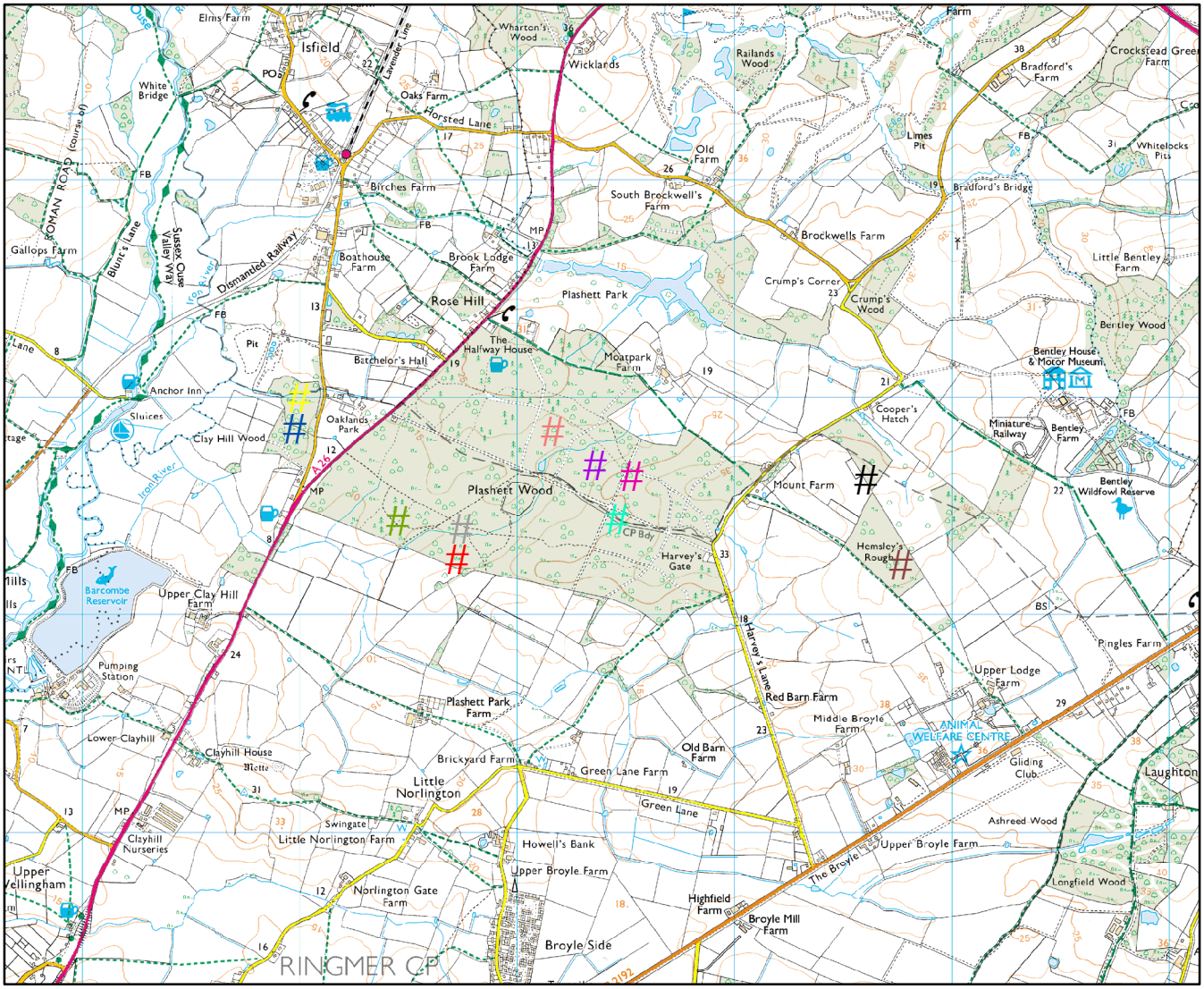
Roost ref		**Date**		**Sunset**		**Sunrise**	
Observer		**Exit hole**		**Moonrise**		**Moonset**	
Temp start		**Temp end**		**Mean max**		**Mean min**	
Humid start		**Humid end**		**Cloud cover**		**Rain**	
Wind		**Time start**		**Time finish**		**Aspect**	
Time of first exit							
Time of last exit							
Time in 10 minute intervals							

	Bats out	**Bats in**	**Total**
1			
2			
3			
4			
5			
6			
7			
8			

Comments

Sketch of exit site plus direction of flight path

Appendix A.3.5: Roost emergence/re-entry recording sheet



0 1 2 Kilometers

Legend

1
2
3
4
5
6
7
8
9
10
11

Appendix A.4.1: Eleven capture locations used at three woodland sites.

Appendix A.4.3: Measuring intensity of the six stimuli

The intensity of the six stimuli was measured in a three stage process. Firstly, the frequency response of the transducer (i.e. the Autobat speaker) was obtained using the following method. A free field microphone (Bruel & Kjaer model 4191 with a flat response up to 40kHz), was placed inside a standard sound source (calibrator Bruel & Kjaer model 4230, 94dB Spl at 1kHz). The microphone's output was amplified by 40dB which was then measured as -1.4dB on a dB meter. However, since the transducer could not be placed directly inside the calibrator, the standard sound source was subsequently moved away from the free field microphone, to a distance of 2cm, which resulted in a reduction of the microphone output to -3.2dB. The standard sound source was then replaced with the transducer at a distance of 2cm and an input from a signal generator (constant level 7.4 volts p-p) was applied while sweeping through the frequency range 1kHz to 60kHz, in 1kHz steps, in order to determine the frequency response of the transducer (See Table A.4.2.1 column 1 for detailed measurements.).

The second stage of the process was to calibrate the microphone of the recording system, the Ultrabat, using the, now calibrated, Autobat transducer as a signal source. The transducer was placed 2cm from the microphone (Knowles EK23132) of the Ultrabat recording system. A constant level signal of 7.4 volts p-p from the signal generator (Venner TSA 625/2) was applied to the transducer. As this level of 7.4 v p-p caused overloading of the recording system, it was reduced by 25dB to prevent overload occurring. This signal was swept through the frequency range of 1khz to 60kHz in 1kHz steps. From the analysis of these recordings it was possible to determine the sound pressure level, (shown in column 2 Table A.4.3.1).

The final stage in the calibration stage was to measure the frequency which produced the highest signal level of the 6 Autobat stimuli calls A – F in Bat Sound. Calls A, D and F were comprised of one call type only, call C comprised of two and calls B and E comprised of three different call types. Once a sound level measurement for the peak frequency was obtained for each of the calls (from the spectrogram in Bat Sound), the Spl that the transducer produced to give this level could be determined using the measurements obtained for the Autobat transducer (Figure A.4.3.1) and Ultrabat recording system calibration (Figure A.4.3.2). So, for example, call A had a peak frequency of 32.7 kHz, -27dB in Batsound. At 33kHz the calibrated microphone gives produces -6dB. The Autobat transducer produces 71.7dB at 33kHz. Therefore, call A at 33kHz produces 50.7 SPL dB (- 6dB less than -27dB is 21dB, 21dB less than 71.7dB gives a reading of 50.7 SPL dB). Amplified calls increased the SPL by – 3dB.

Column A

BK mic (close in)	**SPL (dB)**	**BK mic at 2cm**	**SPL (dB)**
-14	94	-32	76
Frequency (kHz)	**Transducer at 2cm**	**SPL dB**	**SPL dB -25**
1	-53.2	54.8	29.8
2	-50.7	57.3	32.3
3	-44.7	63.3	38.3
4	-41	67	42
5	-35.7	72.3	47.3
6	-32.8	75.2	50.2
7	-30.4	77.6	52.6
8	-28.4	79.6	54.6
9	-26.9	81.1	56.1
10	-25.3	82.7	57.7
11	-25.2	82.8	57.8
12	-24.5	83.5	58.5
13	-22.9	85.1	60.1
14	-22.7	85.3	60.3
15	-23	85	60
16	-22.8	85.2	60.2
17	-22.2	85.8	60.8
18	-21.5	86.5	61.5
19	-21.4	86.6	61.6
20	-21.2	86.8	61.8
21	-20.5	87.5	62.5
22	-17	91	66
23	-15.5	92.5	67.5
24	-14.5	93.5	68.5
25	-15.7	92.3	67.3
26	-15.6	92.4	67.4
27	-15.1	92.9	67.9
28	-13.7	94.3	69.3
29	-9.9	98.1	73.1
30	-10.7	97.3	72.3
31	-10.4	97.6	72.6
32	-11.4	96.6	71.6
33	-11.3	96.7	71.7
34	-10.4	97.6	72.6
35	-9.2	98.8	73.8
36	-7.5	100.5	75.5
37	-8	100	75
38	-8.3	99.7	74.7
39	-8.3	99.7	74.7
40	-8.6	99.4	74.4

Column B

Microphone callibration	
Frequency (kHz)	**SPL Db**
20	-16.2
21	-14.5
22	-13.6
23	-11.1
24	-9.4
25	-6.4
26	-5.1
27	-5.1
28	-7.7
29	-7.7
30	-6.8
31	-1.7
32	-2.6
33	-6
34	-5.5
35	-4.3
36	-1.7
37	-1.7
38	-3.4
39	-4.3
40	-6.1
41	-6
42	-3.4
43	-0.9
44	-3.4
45	-4.3
46	-6
47	-6
48	-2.6
49	-3.4
50	-6
51	-6.2
52	-6.2
53	-3.4
54	-3.4
55	-4.3
56	-4.3
57	-5.1
58	-6
59	-10.2
60	-14.5

CONT....

Frequency (kHz)	Transducer at 2cm	SPL dB	SPL dB -
44	-11.7	96.3	71.3
45	-13.5	94.5	69.5
46	-13.9	94.1	69.1
47	-13.5	94.5	69.5
48	-13.4	94.6	69.6
49	-13.5	94.5	69.5
50	-13.1	94.9	69.9
51	-12.2	95.8	70.8
52	-12	96	71
53	-13	95	70
54	-13.3	94.7	69.7
55	-14.6	93.4	68.4
56	-15.3	92.7	67.7
57	-14.9	93.1	68.1
58	-14.3	93.7	68.7
59	-14	94	69

Table A.4.3: Frequency response of transducer, column 1 and the Ultrabat recording system microphone, column 2.

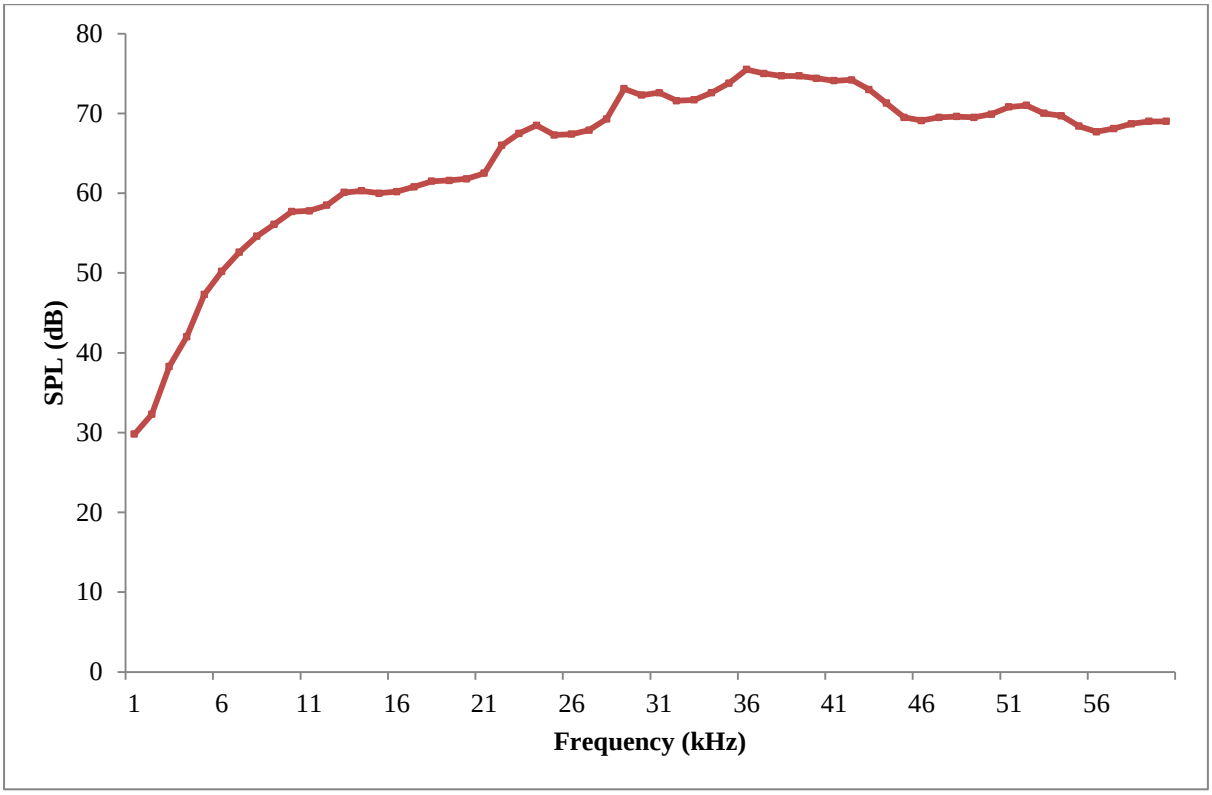


Figure A.4.3.1: Frequency response curve of Autobat transducer.

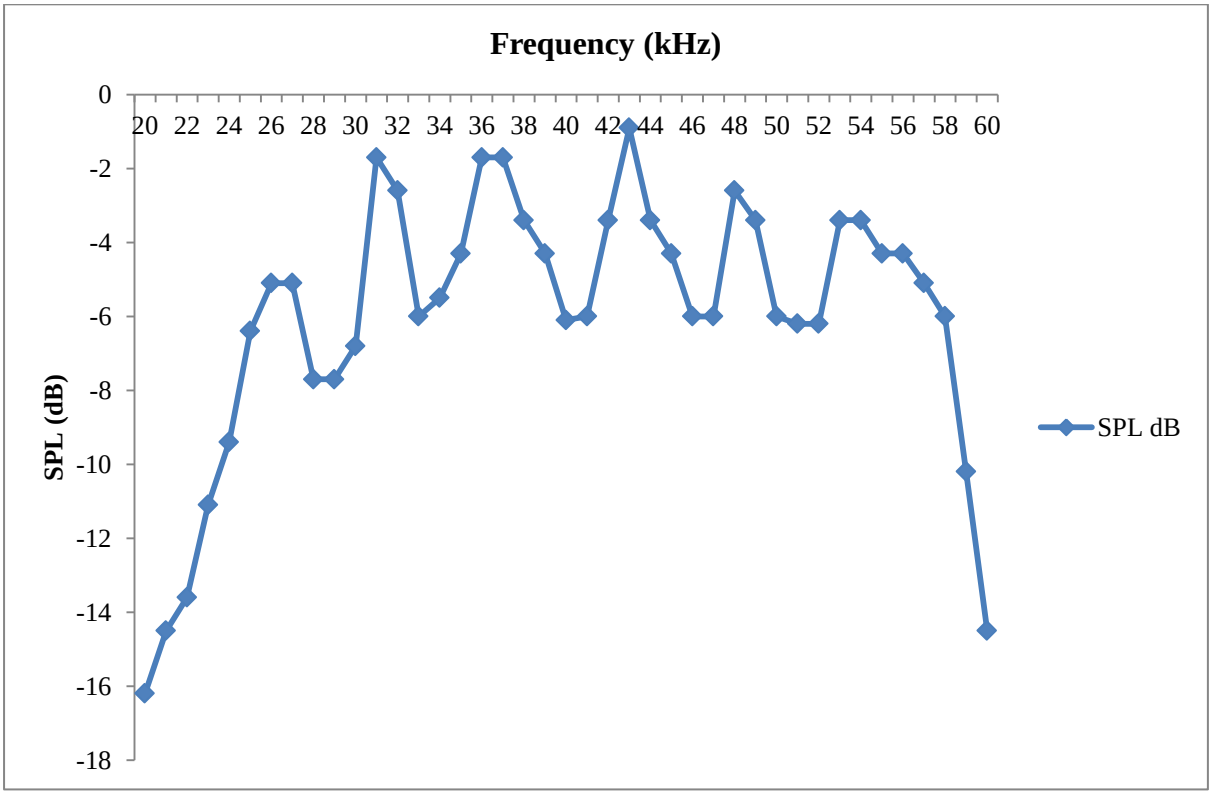


Figure A.4.3.2: Frequency response curve of Ultrabat microphone.

Behavioural definitions for categorisation system

Approach stimulus	The responding bats(s) directly approach the speaker.
Fly near	The responding bats(s) approach the stimulus and subsequently fly near to the stimulus.
Continuous Flight	The responding bat(s) flies in a direct line without deviating from a straight flight path.
Deviated Flight	The responding bat(s) changes direction of flight either on approaching or departing from the stimulus location.
360^{o} rotation	The bat(s) fly around the stimulus in a circular motion completing at least one full 360^{o} rotation.
Moving continuously	The bat(s) continuously flies near to the stimulus without pausing to investigate the sound source and without completing a full 360^{o} rotation.
Hover	The bats(s) pause in their flight to stay suspended in the air fluttering near one place.

Appendix A.4.5: Definitions of terms used in behavioural binary key.

Pair	Bat	Site	MCP 100% (ha)	KDE 95% (ha)	KDE 50% (ha)	% Overlap 100% MCP with simultaneously tracked female	% Overlap 95%KDE with simultaneously tracked female	% Overlap 50%KDE with simultaneously tracked female
1	9	Clayhill	12.9	6.1	2.0	23.4	43.3	55.5
	10	Clayhill	6.4	4.1	2.0	50.8	97.9	87.7
2	13	Hemsleys	5.1	4.0	1.8	55.8	52.4	45.3
	14	Hemsleys	6.1	3.9	2.3	55.9	50.1	42.0
3	17	Capite	7.9	3.1	1.1	0.0	0.0	0.0
	18	Capite	5.1	1.7	1.1	0.0	0.0	0.0
4	19	Bignor	6.1	2.6	1.1	60.3	49.5	38.6
	20	Bignor	4.9	3.1	1.5	65.7	45.6	36.0
5	21	Small Dole	3.9	3.1	1.6	2.4	0.6	0.0
	22	Small Dole	8.5	6.1	3.6	0.9	0.3	0.0
6	23	Small Dole	5.1	4.0	2.0	74.7	58.4	41.8
	24	Small Dole	6.6	4.9	1.9	59.1	59.3	42.3
7	25	Small Dole	8.1	7.5	3.7	52.1	62.1	61.4
	26	Small Dole	6.0	4.1	2.5	38.3	82.7	79.9
8	27	Capite	6.1	3.1	2.1	29.1	24.1	17.5
	28	Capite	7.6	4.0	3.0	24.3	25.1	19.2
9	29	Bignor	5.5	3.5	2.0	35.9	44.0	35.1
	30	Bignor	2.7	1.6	0.7	92.3	95.2	96.8
10	31	Bignor	9.9	6.2	2.8	66.8	49.7	30.0
	32	Bignor	8.9	5.1	3.9	31.4	17.3	18.9

Appendix A.7.1: Percentage overlap of each home range estimate for 20 female *P. auritus* radio-tracked simultaneously in pairs.

Clayhill Wood	Bleb 9	Bleb 10	Bleb 11
Bleb 9		23.40	12.17
Bleb 10	50.80		62.73
Bleb 11	15.78	37.37	

Hemsleys Rough	Bleb 12	Bleb 13	Bleb 14
Bleb 12		30.69	43.59
Bleb 13	70.39		55.80
Bleb 14	83.74	55.90	

Capite Wood	Bleb 17	Bleb 18	Bleb 27	Bleb 28
Bleb 17		0	0.95	35.49
Bleb 18	0		0	0
Bleb 27	1.23	0		29.31
Bleb 28	38.12	0	24.30	

Bignor Park	Bleb 19	Bleb 20	Bleb 29	Bleb 30	Bleb 31	Bleb 32
Bleb 19		60.30	13.04	5.16	13.7	33.79
Bleb 20	65.70		30.27	6.76	5.52	36.16
Bleb 29	13.81	29.65		35.90	58.16	71.9
Bleb 30	10.73	13.14	92.30		93.71	100
Bleb 31	7.42	3.62	29.59	24.29		66.80
Bleb 32	4.13	3.45	8.28	5.87	31.40	

Hoe Wood	Bleb 21	Bleb 22	Bleb 23	Bleb 24	Bleb 25	Bleb 26
Bleb 21		2.40	72.75	92.79	40.99	59.82
Bleb 22	0.90		0	3.72	20.74	3.61
Bleb 23	54.72	0		74.70	37.15	58.39
Bleb 24	55.07	5.12	59.10		52.26	65.58
Bleb 25	19.14	22.46	23.07	41.11		52.10
Bleb 26	38.52	5.39	50	71.17	38.30	

Appendix A.7.2: Percentage range overlap of 100% MCP for simultaneously (no colour) and non-simultaneously (shaded in yellow) radio-tracked bats at the same site in the same year.

Clayhill Wood	Bleb 9	Bleb 10	Bleb 11
Bleb 9		43.30	21.27
Bleb 10	97.90		43.61
Bleb 11	31.24	42.91	

Hemsleys Rough	Bleb 12	Bleb 13	Bleb 14
Bleb 12		26.8	24.88
Bleb 13	36.05		52.4
Bleb 14	42.99	50.1	

Capite Wood	Bleb 17	Bleb 18	Bleb 27	Bleb 28
Bleb 17		0	0.05	7.22
Bleb 18	0		0	0
Bleb 27	0.05	0		24.10
Bleb 28	5.73	0	25.10	

Bignor Park	Bleb 19	Bleb 20	Bleb 29	Bleb 30	Bleb 31	Bleb 32
Bleb 19		49.50	14.79	6.96	18.4	6.47
Bleb 20	45.60		22.11	2.37	0.74	2.96
Bleb 29	11.56	20.56		44.0	42.25	1.28
Bleb 30	5.44	2.19	95.20		33.43	0.75
Bleb 31	8.03	0.38	24.13	18.66		49.70
Bleb 32	2.02	1.1	0.51	0.3	17.30	

Hoe Wood	Bleb 21	Bleb 22	Bleb 23	Bleb 24	Bleb 25	Bleb 26
Bleb 21		0.60	66.82	89.52	43.05	36.37
Bleb 22	0.30		0	3.82	23.25	11.13
Bleb 23	53.64	0		58.4	39.46	34.49
Bleb 24	71.87	4.80	59.3		57.03	48.56
Bleb 25	15.68	17.13	17.90	33.42		62.1
Bleb 26	30.20	18.71	15.64	64.87	82.7	

Appendix A.7.2: Percentage range overlap of 95% KDE for simultaneously (no colour) and non-simultaneously (shaded in green) radio-tracked bats at the same site in the same year.

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Clayhill Wood	Bleb 9	Bleb 10	Bleb 11
Bleb 9		55.50	11.79
Bleb 10	87.70		13.46
Bleb 11	11.31	13.5	

Hemsleys Rough	Bleb 12	Bleb 13	Bleb 14
Bleb 12		5.56	8.17
Bleb 13	8.18		45.30
Bleb 14	14.68	42.0	

Capite Wood	Bleb 17	Bleb 18	Bleb 27	Bleb 28
Bleb 17		0	0	0
Bleb 18	0		0	0
Bleb 27	0	0		17.5
Bleb 28	0	0	19.2	

Bignor Park	Bleb 19	Bleb 20	Bleb 29	Bleb 30	Bleb 31	Bleb 32
Bleb 19		38.60	0.10	0.25	4.61	2.76
Bleb 20	36.0		9.32	0	0	3.47
Bleb 29	0.07	5.59		35.10	20.34	0
Bleb 30	0.80	0	96.80		58.48	0
Bleb 31	2.21	0	13.69	8.80		30.0
Bleb 32	0.97	1.03	0	0	18.90	

Hoe Wood	Bleb 21	Bleb 22	Bleb 23	Bleb 24	Bleb 25	Bleb 26
Bleb 21		0	38.54	42.92	5.18	0
Bleb 22	0		0	0	23.51	2.44
Bleb 23	34.29	0		41.80	72.77	3.67
Bleb 24	27.58	0	42.30		52.54	37.91
Bleb 25	2.38	26.45	1.33	37.55		61.40
Bleb 26	0	6.58	4.53	64.84	79.90	

Appendix A.7.4: Percentage range overlap of 50% KDE for simultaneously (no colour) and non-simultaneously (shaded in blue) of radio-tracked bats at the same site in the same year.